

ANNUAL REPORT

**Division of Intramural Research Programs
National Institute of Mental Health**

October 1, 1987 - September 30, 1988

**VOLUME II PART 1
INDIVIDUAL PROJECT REPORTS**

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration
National Institute of Mental Health
Division of Intramural Research Programs**

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National Institute of Mental Health

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NATIONAL INSTITUTE OF MENTAL HEALTH

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VOLUME II PART I

INDIVIDUAL PROJECT REPORTS

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DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

RESEARCH PROJECT SERIAL NUMBER LISTING:

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ANNUAL REPORT
DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1987 - September 30, 1988

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00183-03 BP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology and Behavior of Aggression and Suicide

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gerald L. Brown, M.D., Medical Officer, BPB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been discontinued as the primary investigator has left the Biological Psychiatry Branch.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 00070-15 BP
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychological and Biological Interactions in the Mood and Anxiety Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Robert M. Post, M.D. Chief BP NIMH Dr. T. Colburn Research Services Branch, NIMH Dr. R. Cohen Laboratory of Cerebral Metabolism, NIMH Dr. L. DeLisi Dept of Psychiatry, State University of N.Y. Dr. T.W. Uhde Biological Psychiatry Branch, NIMH		
COOPERATING UNITS (if any) BPB, CNG, NSB, NPB, CPB, LCM, LCS, LPP, RSB, IRP, NIMH; DEB, NICHD; IRP, NIAAA; PDS, NIH; USUHS, Dept of Def.; U. of CA; Tufts U.; U. So. Carolina Med. Sch.; INSERM; St. Elizabeth's Hosp.; St. Michael's Hosp.; SUNY; U. of Washington; Beth Israel Hosp.		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Psychobiology		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: <div style="text-align: center;">9.0</div>	PROFESSIONAL: <div style="text-align: center;">5.0</div>	OTHER: <div style="text-align: center;">4.0</div>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Evaluation, study, and treatment of patients with <u>manic-depressive</u> and <u>schizoaffective</u> illness are the primary goals of the Section. Double-blind, placebo-controlled clinical trials are employed to evaluate routinely used and novel agents for the treatment of these disorders. <u>Anticonvulsants</u> such as <u>carbamazepine</u> have been demonstrated to be clinically effective in the acute and prophylactic treatment of manic-depressive illness. We have identified possible clinical and biochemical markers of response to <u>lithium</u> versus carbamazepine and other agents. For example, antimanic responders to carbamazepine appear to be more severely ill, more dysphoric, and more rapidly cycling than non-responders, i.e., variables that tend to be associated with lithium nonresponse. In attempting to elucidate possible mechanisms of action, we have found that alpha-2 <u>noradrenergic</u> and "peripheral-type" <u>benzodiazepine receptor</u> mechanisms may be important to the anticonvulsant if not the psychotropic effects of carbamazepine. Other <u>neurotransmitter</u> , <u>modulator</u> , and <u>peptide</u> substances are being studied which may account for carbamazepine's positive effects on mood and behavior. The Section also seeks to identify <u>regional</u> alterations in brain <u>electrophysiological</u> and <u>metabolic</u> activity that are related to changes in <u>behavior</u> and <u>cognition</u> in affective illness. A clinical probe of <u>limbic system</u> excitability utilizing a novel provocative agent, <u>procaine</u> , is also being employed. Procaine selectively increases fast activity over the <u>temporal lobe</u> in association with a variety of behavioral and cognitive alterations and secretion of cortisol, ACTH, and prolactin. Animal models of electrophysiological and pharmacological <u>kindling</u> and cocaine-induced <u>behavioral sensitization</u> are studied and implicate conditioning and learning processes in the progressive behavioral changes induced. These models may help provide new clinical and biochemical insights into the mechanisms that underlie the progressive and long-term changes in behavior in a variety of clinical syndromes including cocaine-induced psychopathology and affective illness.		

COLLABORATORS:

Dr. M.S. Buchsbaum, Dept. of Psychiatry, U. of California, Irvine
 Dr. D.C. Jimerson, Dept. of Psychiatry, Beth Israel Hospital, Boston
 Dr. F.K. Goodwin, Office of the Director, IRP, NIMH
 Dr. P.W. Gold, Clinical Neuroendocrinology Branch, NIMH
 Dr. M. Linnoila, Intramural Research Program, NIAAA
 Dr. H. Weingartner, Biological Psychiatry Branch, NIMH
 Dr. D.R. Rubinow, Biological Psychiatry Branch, NIMH
 Dr. C.H. Kellner, Dept of Psychiatry, U. of So. Carolina Medical School
 Dr. R. Coppola, Neuropsychiatry Branch, St. Elizabeth's Hospital
 Dr. R. Cowdry, Research Services Branch, St. Elizabeth's Hospital
 Dr. D. Gardner, Neuroscience Branch, NIMH
 Dr. S.R.B. Weiss, Biological Psychiatry Branch, NIMH
 Dr. A. Pert, Biological Psychiatry Branch, NIMH
 Dr. P. Marangos, Research Division, Gensia Pharmaceuticals, San Diego
 Dr. J. Patel, Dept. of Pharmacology, Stuart Pharmaceuticals
 Dr. S. Reichlin, Division of Endocrinology, Tufts University
 Dr. J.C. Ballenger, Dept. of Psychiatry, U. of So. Carolina Medical School
 Dr. F. Putnam, Neuropsychiatry Branch, NIMH
 G. Leverich, Biological Psychiatry Branch, NIMH
 Dr. S.L. Brown, Lab. of Developmental Psychology, NIMH
 Dr. P. Roy-Byrne, Dept. of Psychiatry, Univ. of Washington
 Dr. M. Kling, Clinical Neuroendocrinology Branch, NIMH
 Dr. D. Davis, Biological Psychiatry Branch, NIMH
 Dr. K. Kramlinger, Dept. of Psychiatry, Mayo Clinic
 Dr. K. Denicoff, Biological Psychiatry Branch, NIMH
 Dr. T. Melman, Biological Psychiatry Branch, NIMH
 Dr. J-P. Boulenger, French National Institute for Health and Med. Res. (INSERM),
 Cannes, France
 Dr. R. Joffe, Dept of Psychiatry, St. Michael's Hospital, Toronto, Canada
 Dr. A.F. Mirsky, Lab. of Psychology and Psychopathology, NIMH
 Dr. G. Chrousos, Developmental Endocrinology Branch, NICHD
 Dr. B. Jones, Lab. of Psychology and Psychopathology, NIMH
 Dr. C.D. Johnson, Lab. of Psychology and Psychopathology, NIMH
 Dr. A. Doran, Dept. of Psychiatry, Univ. of California at Davis
 Dr. D. Pickar, Neuroscience Branch, NIMH
 A. Rosoff, Biological Psychiatry Branch, NIMH
 Dr. P. Hauser, Biological Psychiatry Branch, NIMH
 Dr. S. Paul, Neuroscience Branch, NIMH
 Dr. A. Roy, Intramural Research Program, NIAAA
 Dr. L. Altshuler, Biological Psychiatry Branch, NIMH
 Dr. A. Calogero, Developmental Endocrinology Branch, NICHD
 Dr. O. Devinsky, Epilepsy Branch, NINCDS
 Dr. E. Bromfield, Epilepsy Branch, NINCDS
 Dr. J.C. Daval, Biological Psychiatry Branch, NIMH
 Dr. T. Nakajima, Biological Psychiatry Branch, NIMH
 Dr. M. Casanova, Clinical Brain Disorders Branch, St.-Elizabeth's Hospital

I. Project Description

A. Objectives

This project is engaged in the multidisciplinary longitudinal study and treatment of patients with a spectrum of acute and chronic psychoses, particularly involving mood and anxiety disorders. Both investigative and treatment approaches focus on the elucidation of psychological and biological phenomena and their interaction.

B. Methods Employed

1. Subjects who meet Research Diagnostic Criteria (RDC) for manic-depressive or schizoaffective illness or the more recent DSM III criteria for a spectrum of mood disorders are admitted to the 3-West Clinical Research Unit, Section on Psychobiology of the Biological Psychiatry Branch. Patients with anxiety and panic-anxiety are also admitted to the unit under other protocols (see Project Z01 MH 00071-08 BP). Normal volunteers are admitted to the unit to provide control data for specific studies in patients and to assess clinical and biological interrelationships in normal as well as patient populations.

2. Behavioral and Psychological Evaluation

a. Psychological Evaluation: Patients are rated twice daily in a double-blind fashion and are assessed with a variety of psychological tests (as previously described). Life course of illness is charted graphically in great detail.

b. Biological Evaluation: Sleep EEG, AER, and glucose utilization on PET are studied during medication-free intervals, as is procaine activation of EEG, behavior, and cognition. Neurotransmitters, endocrine substances, and peptides are measured in urine, plasma, and CSF before and after acute drug challenges and longer-term treatment. Endocrine tests are described in project #Z01 MH 00452-13 BP.

3. Treatment

Drug evaluation is conducted in a double-blind fashion. Routinely employed drugs include lithium, neuroleptics, tricyclic and MAOI antidepressants. New and experimental treatments include carbamazepine, carbamazepine-10,11-epoxide, valproic acid, clonazepam, phenytoin, clonidine, sleep deprivation, l-baclofen, verapamil, and T_3 or T_4 potentiation.

4. Animal Models

A rodent behavioral pharmacology laboratory is maintained in collaboration with Drs. S.R.B. Weiss and A. Pert to develop new research techniques in several areas. The longitudinal evolution of behavioral pathology and its underlying biochemical mechanisms are assessed in different paradigms including: 1) electrophysiological kindling; 2) pharmacological kindling; and 3) behavioral sensitization to psychomotor stimulants such as cocaine. The role of seizures in the development of behavioral pathology is studied utilizing electrical and pharmacological kindling and CRF. The anticonvulsant mechanism of action of carbamazepine is also studied in the kindling paradigm.

C. Major Findings

1. Carbamazepine: A New Treatment and an Alternative or Adjunct to Lithium for Manic-Depressive Illness

a. Prophylactic Efficacy of Carbamazepine: We have followed 24 patients during long-term carbamazepine prophylaxis for an average of four years (range 1-10 years). Essentially all of these patients were nonresponsive or poorly responsive to lithium carbonate, the traditional treatment for manic-depressive illness. Total numbers of episodes per year were reduced by approximately 50% following the use of carbamazepine, either alone or in combination with lithium carbonate, in the majority of patients. An illness index was constructed by multiplying severity (.25 = mild, .5 = moderate, and 1.0 = severe incapacitation) and duration of an episode. Over the four baseline years prior to the onset of carbamazepine treatment, the average illness index progressively deteriorated from 13 to 22 in the year prior to beginning carbamazepine treatment. An illness index of 22 reflects essentially 22 weeks of severe incapacitation or hospitalization or 44 weeks at a moderate level of severity, etc. During treatment with carbamazepine, the illness index decreased to the range of 6-8 or below during each year of the follow-up. Half the patients followed for more than two years showed a pattern of persistent suppression of episodes and affective morbidity on the illness index (N=11 subjects). However, the other half of the subjects (N=11) showed a pattern of loss of efficacy with reemergence of episodes and an increase on the illness index during the second or third year of carbamazepine treatment. These data suggest that patients followed during carbamazepine prophylaxis in the community may in some instances begin to show a loss of efficacy. Whether people with this pattern of illness would respond adequately to carbamazepine dose increases or other pharmacological interventions remains to be more systematically tested, along with the factors that may be causally related to this problem. We are undertaking studies to assess whether there is a similar loss of efficacy in the subgroup of patients treated with long-term lithium carbonate. In preclinical models (discussed below) we have elucidated the novel phenomenon of conditioned tolerance to the anticonvulsant effects of carbamazepine on amygdala-kindled seizures in the rat. Further investigations will be aimed at addressing whether a similar phenomenon of conditioned tolerance is occurring to the psychotropic effects of carbamazepine in manic-depressive illness, in that patients who are initially highly responsive to the drug begin to show a reemergence of episodes over time.

b. Acute Antimanic Efficacy: In collaboration with Drs. T. Uhde, K. Kramlinger, and other physicians in the Branch, we have found that carbamazepine is effective in the acute treatment of manic patients, including many who were previously nonresponsive to lithium carbonate. The magnitude and time-course of improvement on carbamazepine paralleled that of neuroleptics. Sleep improved significantly in the first week of treatment. Twelve of 19 (63%) acutely manic patients have shown good responses. These responders, compared with nonresponders, were more severely manic and dysphoric at the onset during their placebo period and they were also more rapid cyclers. Responders had a negative family history for manic-depressive illness in first degree relatives, while nonresponders were equally divided among family history positives and negatives. These data suggest an opposite clinical profile of response to lithium and car-

bamazepine. While manic severity, dysphoria, rapid-cycling, and negative family history tend to be associated with poorer response to lithium, these variables are associated with better antimanic response to carbamazepine.

Potentiation of carbamazepine with the addition of lithium carbonate resulted in improvement in five of six patients who had previously been inadequately responsive to the antimanic effects of either drug alone.

c. Acute Antidepressant Efficacy: Fifteen of the first 47 patients have shown evidence of a marked clinical response to carbamazepine. Patients with initially more severe depression responded better to carbamazepine than those with less severe ratings of depression. Those with more rapid cycling (episodes/years ill) and hospitalizations for mania, but fewer total weeks depressed (i.e., less chronic depression), also responded better (see also thyroid correlate of response below).

In 15 depressed patients who were inadequately responsive to the acute antidepressant effects of carbamazepine alone administered on a double-blind basis, lithium was added, also on a blind basis (with K. Kramlinger). Eight of the 15 patients showed a marked response to this lithium potentiation. The time-course of acute antidepressant response was rapid, more so than previously observed in responders to lithium treatment alone. These data suggest that lithium potentiates the antidepressant effects of carbamazepine, as has been reported for many other antidepressant modalities, including heterocyclics and monoamine oxidase inhibitors. While the mechanism of action remains to be elucidated, the rapid onset of lithium potentiation of the more traditional antidepressants has been suggested by de Montigny to involve the serotonin system. Not only was the time course of acute antidepressant response to lithium potentiation of carbamazepine faster than the antidepressant response achieved with either agent alone, but it was also faster than the onset of the antimanic response to lithium potentiation. These data further support the concept that the rapid onset of acute antidepressant effects with lithium potentiation may be achieved by biological mechanisms that are different from those engaged in the antimanic effects or those attributable to either drug alone.

d. Side Effects of Carbamazepine-Lithium Combination Treatment: The side-effects profiles of carbamazepine and lithium tend to be substantially different, offering the patient the possibility of alternative treatment should one drug or the other not be well tolerated. Problems often observed with lithium (thirst, polyuria, tremor, weight gain, cognitive slowing, and hypothyroidism) tend not to occur with carbamazepine treatment, which is associated with its own profile of side effects. These include ataxia, dizziness, fatigue, and diplopia, which are dose-related and can be avoided with slow upward titration of dose.

When the two drugs are used in combination, important interactions are observed. Carbamazepine alone decreases white count in the majority of patients, while lithium increases white count. During lithium potentiation of carbamazepine treatment of 22 depressed or manic patients, Dr. Kramlinger observed that lithium reversed the white count suppression induced by carbamazepine and by

the third week of combination treatment, values were actually increased over those observed during the baseline placebo period. The degree of carbamazepine-induced decrease was correlated with the degree of lithium-induced increase. These data are consistent with recent observations by Gallicchio that carbamazepine suppresses and lithium enhances bone marrow colony-stimulating factor for granulocytes.

Lithium induces diabetes insipidus while carbamazepine has been used to treat the syndrome. However, because the effects of lithium carbonate occur at a level beyond the receptor, probably involving adenylate cyclase, carbamazepine is not able to override the effects of lithium and will not reverse lithium-induced diabetes insipidus. While carbamazepine induces mild decreases in serum sodium and calcium, these effects were not significantly reversed by lithium potentiation. Rarely, carbamazepine is associated with hyponatremia and water intoxication.

Carbamazepine decreases plasma levels of T_4 , free T_4 , and T_3 without significantly increasing TSH. Lithium potentiation results in further decreases in circulating thyroid hormone levels and increases in TSH secretion. These data suggest that lithium and carbamazepine are impairing thyroid hormone levels at different steps in the regulation of thyroid function. Other data suggest that carbamazepine may increase peripheral thyroid metabolism.

In contrast to lithium carbonate, which can induce clinical hypothyroidism that requires supplemental treatment with thyroid hormone in a small but substantial percentage of patients, hypothyroidism on carbamazepine is rarely induced and has not been observed in our series. In fact, those with the greatest decrements in circulating T_4 and free T_4 have shown the greatest degree of acute antidepressant response to carbamazepine. These paradoxical data are consistent with studies of Baumgartner and associates, indicating that patients with greater decrements in thyroid indices on maprotiline and zimelidine respond better to these treatments.

Rashes have been observed in 13 of the first 113 patients (11.7%) and carbamazepine was discontinued in each instance. The rashes were uniformly pruritic in nature and, in 12 instances, occurred in the second or third week of carbamazepine treatment. One rash became exfoliative.

e. Carbamazepine and its -10,11-Epoxy Metabolite: Levels of carbamazepine itself in plasma or in CSF do not appear to be related to the degree of clinical antidepressant or antimanic response. However, preliminary data suggest that the levels of the active metabolite of carbamazepine, carbamazepine-10,11-epoxide, measured in CSF, were more closely related to the degree of antidepressant response. This metabolite has been demonstrated by us to have anticonvulsant effects on amygdala-kindled seizures and by others to have antinociceptive properties and to be effective in the treatment of trigeminal neuralgia. Based on these data, we have undertaken a clinical trial of carbamazepine-10,11-epoxide in order to establish whether it has psychotropic properties. While the first patient entered in the clinical trial did not respond at low blood levels to the 10,11-epoxide for the treatment of acute mania, a second

patient showed excellent maintenance of therapeutic response when switched from carbamazepine to its metabolite, suggesting possible efficacy of the epoxide in affective illness.

f. Selective Responses to Different Anticonvulsant Agents in Affectively Ill Patients: Response to one anticonvulsant does not appear to produce response to another. We have observed clearcut response to carbamazepine but not to valproic acid or phenytoin in an individual patient completing a double-blind, crossover to these three agents. Conversely, we have observed other patients who are inadequately responsive to carbamazepine but who respond to valproic acid. These data are not only of clinical import, but suggest the possibility that differential biochemical or physiological properties of different anticonvulsants may be related to differential clinical responsivities in different patients. This may be particularly apparent in the case of carbamazepine compared with clonazepam, where the two drugs exert differential effects on benzodiazepine receptors; carbamazepine likely interacting with the "peripheral-type" benzodiazepine receptor and clonazepam acting exclusively at the "central-type". We are also beginning to explore the utility of combination treatment in refractory bipolar patients. A patient with a 30-year history of ultra-rapid cycling manic-depressive illness responded well only after T_3 (50 μ g) had been added to the combination of carbamazepine, lithium, and valproic acid.

The effectiveness of the anticonvulsants carbamazepine, valproate, clonazepam, and related drugs raises the paradox of why both anticonvulsants and the induction of seizures with electroconvulsive therapy (ECT) are useful treatments for acute manic and depressive illness. We have observed that electroconvulsive seizures in the rat are paradoxically anticonvulsant to amygdala-kindled seizures. These data raise the possibility that common biochemical and physiological mechanisms of electroconvulsive therapy and anticonvulsants such as carbamazepine could be related to their profile of therapeutic efficacy in both phases of affective illness.

g. Studies of Carbamazepine's Mechanism of Action:

1) Effects on Classical Neurotransmitters and Modulators:

Evidence of others in laboratory animals suggests that carbamazepine blocks the reuptake of norepinephrine (NE), but also inhibits stimulated release; it increases firing of the locus coeruleus, but decreases NE turnover. Elevated levels of CSF NE in mania are decreased by carbamazepine.

Evidence from our laboratory suggest that noradrenergic effects of carbamazepine are important to its anticonvulsant properties. An α -2 mechanism is likely involved in the anticonvulsant effects, as the α -2 antagonist, yohimbine, reverses the effects of carbamazepine on amygdala kindling. α -2 effects may be necessary but not sufficient for the anticonvulsant effects, as the α -2 agonist clonidine is not an effective anticonvulsant on this model of kindled seizures.

Although it is as effective as the neuroleptics in the treatment of acute mania, carbamazepine does not block dopamine receptors or produce other typical

neuroleptic effects. Moreover, it has not been associated with the development of parkinsonian side effects or with the syndrome of tardive dyskinesia as have the neuroleptic drugs. These data suggest that carbamazepine acts by mechanisms other than blockade of dopamine receptors.

Alterations in GABA have been postulated in affective illness (see below) as well as in the seizure disorders. Carbamazepine has been reported to decrease the turnover of GABA in animal studies (Bernasconi, 1984), although brain levels are not altered by the drug. This is consistent with our data indicating that CSF GABA levels are not significantly decreased during treatment with carbamazepine compared with baseline levels.

GABA-B (baclofen type) mechanisms are implicated in the antinociceptive actions of carbamazepine based on animal models of trigeminal neuralgia. For example, Terrence et al (1983) reported that an inactive isomer, d-baclofen, reversed the antinociceptive effects of carbamazepine and the active isomer l-baclofen. In contrast, we have demonstrated that the anticonvulsant effects of carbamazepine on amygdala kindling do not appear to involve GABA-B mechanisms, as these effects are not reversed by d-baclofen.

Thus, it remains to be determined whether the effects of carbamazepine in manic-depressive illness are more akin to those in trigeminal neuralgia (potentially involving GABA-B mechanisms) or in seizures (such as amygdala kindling that do not involve GABA-B mechanisms). In order to assess these differential possibilities, we have undertaken a clinical trial of the active isomer, l-baclofen, in the treatment of manic-depressive patients. Two patients did not respond to l-baclofen in doses up to 10 mg/day (the initial limit imposed by the FDA), and a third has not responded at a dose of 20 mg/day. Further patients will be tested at higher doses when FDA approval is obtained. Most effective antidepressants have been reported to increase GABA-B receptors in frontal cortex (Lloyd et al, 1987), giving further rationale to the baclofen trial, although our preliminary data does not suggest the efficacy of direct GABA-agonist treatment in depression.

Effects of carbamazepine on central and "peripheral-type" benzodiazepine receptors have been studied with biochemical techniques (Marangos et al), and electrophysiologically in the amygdala kindling model (Weiss et al). Carbamazepine binds poorly to the central site (^3H -diazepam or ^3H -BCC), but more potently at the Ro5-4864 (peripheral) site. Dr. S.R.B. Weiss has found that Ro-15-1788 and BCCM block the anticonvulsant actions of diazepam, but are ineffective in reversing the anticonvulsant effects of carbamazepine on amygdala kindling. Conversely, Ro5-4864 does reverse the anticonvulsant effects of carbamazepine and its 10,11-epoxide, but not those of diazepam. PK-11195, which acts selectively at the peripheral site, blocks the effects of Ro5-4864 on carbamazepine's anticonvulsant effects. Taken together, these biochemical and electrophysiological data suggest that carbamazepine may exert its anticonvulsant effects through the "peripheral-type" but not the "central-type" benzodiazepine receptor, and thus act very differently from diazepam and clonazepam. It is of interest that "central-type" benzodiazepine receptors are linked to chloride channels, while the "peripheral-type" may be linked to calcium channels.

Carbamazepine is potent in displacing binding of adenosine receptor ligands (Marangos et al). Contrary to predictions, chronic treatment with carbamazepine (similar to that with caffeine) increased the number of adenosine receptors, suggesting that carbamazepine may possess adenosine antagonist-like properties. It does not appear that carbamazepine exerts its anticonvulsant effects through the adenosine receptor, as its effects on kindled seizures are not altered by several adenosine-active agents (S.R.B. Weiss). In contrast to caffeine, the increase in adenosine receptors following carbamazepine treatment was long lasting (possibly permanent) following chronic administration, and persistent following a single dose, suggesting a novel mechanism for this effect. In a receptor autoradiography study, carbamazepine and caffeine upregulated adenosine receptors in similar areas of brain and also altered second messenger systems.

2. Carbamazepine's Effects on Endocrine and Peptide Systems

Carbamazepine significantly decreased somatostatin measured in CSF of affectively ill patients (studied in collaboration with Drs. D.R. Rubinow, P.W. Gold, and S. Reichlin). These findings, which have recently been replicated in neurological patients (Steardo et al, 1986), are of interest in relationship to the reports by others of long-lasting increases in brain somatostatin following amygdala-kindled seizures and observations that depletions of somatostatin exert anticonvulsant effects on kindled and CRF seizures. Thus, changes in somatostatin could relate to the anticonvulsant properties of carbamazepine.

Carbamazepine may directly or indirectly potentiate vasopressin effects at the receptor level. Rubinow and associates have found that carbamazepine induces escape from dexamethasone suppression and increases urinary free cortisol excretion. Carbamazepine inhibits local-anesthetic-induced release of CRF (Calogeras, Gold, and associates), a finding that is difficult to juxtapose with its ability to increase UFC, but which may be important to its ability to block cocaine-kindled seizures and to other components of its unique spectrum of clinical efficacy in various neuropsychiatric disorders. Carbamazepine did not alter CSF opiate binding activity in affectively ill patients, or affect morphine's antinociceptive effects on tail flick latencies in the rat. In contrast to lithium, carbamazepine inhibits rather than potentiates the TSH response to TRH.

3. Physiological and Behavioral-Pharmacological Effects of Carbamazepine

a. Efficacy as a Function of Stage of Kindling: The anticonvulsant effects of carbamazepine have been examined in several different types of kindling paradigms studied in collaboration with Dr. S.R.B. Weiss. Remarkable dissociations in the anticonvulsant efficacy of carbamazepine have been revealed as a function of stage and type of kindling. For example, carbamazepine is ineffective in blocking the development of electrical kindling of the amygdala in the rat, even though it is highly potent in blocking completed amygdala-kindled seizures. Conversely, carbamazepine blocks the development of lidocaine- and cocaine-kindled seizures, but acutely is ineffective against completed or high-dose local anesthetic seizures. Pinel (1983) has demonstrated that spontaneous seizures which occur after many hundreds of kindled seizures are also

differentially pharmaco-responsive compared with the early stages of kindling. Thus, there appears to be a general principle that different stages in the evolution of kindling -- developmental, completed, and spontaneous -- may be differentially responsive and, therefore, involve different neuroanatomical, physiological, and/or biochemical substrates. Different types of kindling are also differentially responsive.

b. Acute Effects on Amygdala Kindling: The bulk of our work has utilized completed kindled seizures in order to elucidate possible mechanisms of anticonvulsant effects of carbamazepine. Our data implicate noradrenergic α -2 mechanisms and "peripheral-type" benzodiazepine receptor mechanisms in this effect. Data from other investigators also suggest that stabilization of type-2 sodium channels are likely involved in the anticonvulsant effects of both carbamazepine and phenytoin.

However, our time course analysis of the clinical efficacy of carbamazepine in epilepsy, pain syndromes, and affective illness suggests that the mechanisms underlying the anticonvulsant effects of carbamazepine, which occur almost immediately, may be different from those underlying efficacy in mania and depression, which require some 2-3 weeks before they become fully manifest. Based on this analysis, one would want to investigate anticonvulsant mechanisms that require time to develop in order to have a more parallel model for the time frame of efficacy occurring in manic-depressive illness. The effects of carbamazepine on cocaine-induced seizures described below appear to be a useful paradigm in this regard.

c. Inhibition of the Development of Cocaine and Lidocaine Kindling: In contrast to electrical kindling of the amygdala, which is not responsive to carbamazepine in the early developmental stage, the development of pharmacological kindling with local anesthetics is robustly inhibited by carbamazepine. Carbamazepine almost completely blocks the development of lidocaine-kindled seizures and also is highly effective in blocking the development of cocaine-kindled seizures in three separate studies at doses of 40, 50, and 65 mg/kg, respectively. At these doses, animals begin to rapidly develop seizures to a dose of drug that was previously non-convulsive and, in contrast to lidocaine seizures which are well tolerated, those associated with cocaine are extremely lethal. Carbamazepine not only blocks the development of cocaine-kindled seizures, but markedly reduces its associated lethality. In spite of this robust effect on the development of local-anesthetic-induced kindling, carbamazepine is ineffective in blocking completed lidocaine-kindled seizures or high-dose cocaine seizures following acute carbamazepine administration.

d. Requirement for Chronic Administration of Carbamazepine in Local Anesthetic Kindling: While chronic oral administration of carbamazepine in the diet is highly potent in blocking the development of cocaine- and lidocaine-induced pharmacological kindling, we have observed that chronic intermittent administration of the drug is insufficient to produce this effect. When animals are pretreated with carbamazepine (15 mg/kg i.p.) prior to local anesthetic injection on a once-daily basis, animals develop local anesthetic-induced seizures at the same rate as vehicle controls. That is, there is no

effect of chronic intermittent administration of carbamazepine in doses that are highly effective in inhibiting amygdala-kindled seizures. These new data are not only important in their own right, but suggest that the biochemical effects of chronic carbamazepine are not only different from those of acute administration of the drug, but also different from those produced by a chronic intermittent dosage schedule. In addition, these data may be important from an additional clinical perspective. In contrast to some seizure models and trigeminal neuralgia, where carbamazepine appears effective acutely in a matter of days, efficacy in mania and depression often lag several weeks or more. Therefore, it would appear that the effects of carbamazepine in manic-depressive illness may be more closely associated with a seizure model in which chronic drug administration is required in order to demonstrate efficacy. Direct comparisons of the biochemical effects of acute compared with chronic and also compared with chronic intermittent administration should, thus, provide a preliminary means for separating biochemical effects, which may be differentially related to the differential onsets of efficacy in different neuropsychiatric syndromes.

e. Clinical Implications: Lithium carbonate is ineffective in blocking either the development of amygdala-kindled seizures or completed kindled seizures, yet appears to have some efficacy in blocking cocaine-induced behavioral sensitization (described below). Conversely, carbamazepine is a highly effective anticonvulsant for some types of kindled seizures, but is without effect in blocking cocaine-induced behavioral sensitization. Thus, comparison of the effects of lithium and carbamazepine not only on a biochemical but also on a physiological and behavioral/pharmacological basis may help to elucidate different mechanisms of action of these two compounds, which are both effective in manic-depressive patients.

The amygdala-kindling data suggest that different stages in the development of epilepsy will also show differences in anticonvulsant responsivity. One might ask whether a similar principle exists for treatment of different stages in the evolution of affective illness. A considerable body of data suggest that lithium carbonate is less effective in the rapid or continuous cycling type of illness, which often occurs late in the course of affective illness, while these patients may be among those who respond best to carbamazepine. Thus, in addition to a variety of other characteristics that may differentiate lithium responders from carbamazepine responders, work with the animal models suggests the utility of considering stages in the developmental longitudinal course of the illness as a relevant variable, with the possibility emerging that carbamazepine and related anticonvulsants may be most useful in the later stages of affective illness, which tend to be less responsive to treatment with lithium carbonate.

f. Conditioned Inefficacy to Carbamazepine and its Reversal: In collaboration with Dr. S.R.B. Weiss, we have, for the first time, elucidated the phenomenon of conditioned inefficacy to an anticonvulsant drug and its reversal by mechanisms apparently related to deconditioning. We have made use of the fact that carbamazepine will not block the development of amygdala-kindled seizures in the rat. If animals are repeatedly given carbamazepine

(15 mg/kg i.p.) before each kindled seizure during development of amygdala kindling, once these animals enter a phase of completed kindled seizure development, which would ordinarily be highly responsive to the anticonvulsant effects of carbamazepine, they remain refractory. In contrast, in a control group that receives identical injections of carbamazepine and amygdala-kindled stimulation but, in each instance, receives the drug immediately after the kindled seizure, the animals are highly responsive to the anticonvulsant effects of carbamazepine on amygdala-kindled seizures. In addition, animals not treated with carbamazepine at all during amygdala kindling development are also highly responsive to carbamazepine on the completed kindled seizures. Thus, these data suggest that inefficacy to carbamazepine's anticonvulsant effect is conditioned by the animal's prior exposure to carbamazepine during the kindling process, as opposed to animals that receive carbamazepine after they were kindled, or naive controls. Further support for this hypothesis is gleaned by the results of our reversal studies which demonstrate that repeated deconditioning by administering carbamazepine after amygdala-kindled seizures or kindling animals without drug will be sufficient to partially reverse the conditioned inefficacy. In contrast, waiting for an extended period of time or administering carbamazepine without kindled seizures is insufficient to reverse the conditioned inefficacy. These data suggest the possibility that treating animals (and perhaps also patients) with anticonvulsant agents during a phase when they are ineffective, may preclude their later effectiveness during a different phase of the seizure disorder process.

g. Conditioned Tolerance to the Anticonvulsant Effects of Carbamazepine: If animals that are showing a good acute anticonvulsant effect of carbamazepine on amygdala-kindled seizures are repeatedly given the drug on a once-daily basis prior to kindled seizures, eventually tolerance to the anticonvulsant effects will develop. This, too, appears to be conditioned, as it will be reversed by a period of treatment with carbamazepine after kindled seizures occur or by a period of treatment with kindled seizures without drug. In contrast, once animals have developed conditioned tolerance, waiting as long as three weeks without drug or kindling treatment will not be sufficient to alter the conditioned tolerance. Treatment with carbamazepine alone for 11 days is likewise insufficient. Thus, it appears that the occurrence of seizures in the absence of drug is required to reverse the occurrence of conditioned tolerance. These data may have direct clinical implications for epileptic patients who, in some instances, begin to develop seizures to a drug that was previously effective. Renewed efficacy may be obtained for a brief period of time following a period of medication withdrawal, although unfortunately, our pre-clinical data would suggest that the reemergence of seizures may also be required to reverse the conditioned tolerance. While increasing the dose of carbamazepine may help transiently to regain anticonvulsant efficacy, initial studies suggest that repeated treatment with high doses of carbamazepine (25 mg/kg i.p.) leads to the development of tolerance as fast as when animals receive conventional doses of the drug (15 mg/kg i.p.). Clearly, further investigation is warranted in order to assess possible manipulations that would retard and/or reverse the development of conditioned tolerance. Conditioned tolerance appears to be drug-specific, as animals that had developed it to carbamazepine will show an anticonvulsant response to a different agent of the benzodiazepine class, such as diazepam.

The phenomenon of conditioned tolerance may also have implications for patients with trigeminal neuralgia, many of whom develop increasing nonresponsiveness to carbamazepine after initial periods of efficacy. As described above, some of our patients with manic-depressive illness who are showing excellent initial responses to the drug, begin to also show the development of emergence of episodes. The data on conditioned tolerance to the anticonvulsant effects of carbamazepine raise the possibility that a similar phenomenon could be occurring in affectively ill patients and that a period of time off medication may be required in order to demonstrate renewed efficacy to the drug. Clearly, these highly preliminary preclinical suggestions remain to be directly confirmed in the clinic, but do appear to offer novel interpretations for data suggesting that a subgroup of patients may begin to develop tolerance to the psychotropic effects of carbamazepine.

4. Approaches to Classical Neurotransmitter and Peptide Dysfunction in Affective Illness

a. CSF norepinephrine (NE) is significantly increased in manic patients compared with either of the other patient or control populations. Indirect biochemical, pharmacological, and endocrine data continue to suggest a role for dopamine in some aspects of affective illness. Dopamine and its metabolite HVA and DOPAC are studied, in collaboration with Drs. D. Rubinow and M. Linnoila, in the CSF of depressed, manic, and euthymic patients and controls. Studies of the relationship of plasma HVA to the longitudinal course of affective illness suggest only weak relationships to mood or anxiety in the rare individual patient, but no consistent relationship like those reported for plasma HVA and severity of psychosis in schizophrenics.

b. Dr. Rubinow found that CSF somatostatin is significantly decreased in depressed patients compared with those re-studied in the euthymic state or compared with normal volunteer controls. There have now been eight replications in other laboratories of the finding of low somatostatin in depressives compared with normals or other psychiatric comparison groups. These findings are of interest in relationship to the reports of decreased somatostatin in brain and CSF of patients with Alzheimer's disease and several other neuropsychiatric syndromes that can present with cognitive defects, including multiple sclerosis and parkinsonism. Dr. Rubinow, in collaboration with Drs. A. Doran, D. Pickar, A. Roy, and S. Paul, has also found that depressed and schizophrenic patients who were cortisol hypersecretors, as indicated by escape from dexamethasone suppression, had significantly lower CSF somatostatin. The causal links in the relationship are unclear, as somatostatin and cortisol can each influence the other; for example, glucocorticoid treatment of normal volunteers decreases CSF somatostatin (Wolkowitz & Rubinow).

Dr. Gold has completed a series of studies of CRH infusions in affectively ill patients and controls (as described in Project # Z01 MH 00452-13 BP) and found evidence for blunted ACTH response in depressive but not manic or improved states. In contrast to depressed patients, hypercortisolemic patients with Cushing's disease show ACTH hypersecretion to CRF, providing a possible differential diagnostic test.

5. Course of Affective Illness: Relationship to Biochemical and Clinical Variables

We have characterized the course of more than 100 affectively ill patients' on our clinical research unit over the past several years. These descriptive data are of considerable interest in their own right, but also form a critical substrate for analyzing the relationship of the course of illness to subsequent pharmacological response (see data on carbamazepine above) as well as to various neurobiological alterations observed in the illness. An example of this usefulness is derived from our recent study of the relationship of thyroid dysfunctions in manic-depressive illness. It had previously been reported by others that patients with hypothyroidism were overly represented in a group with rapid cycling.

a. Thyroid Function and Course of Illness: In order to more systematically examine this question, we assessed thyroid indices at several points during the NIMH hospitalization in relationship to retrospective and prospective course-of-illness variables. Values were obtained at the onset and termination of the medication-free observation interval at NIMH and during treatment with both lithium and carbamazepine. Thyroid values changed from the first to the second medication-free period in a highly consistent fashion. T_4 and free T_4 levels increased while T_3 levels decreased, suggesting decreased conversion of T_4 to T_3 consistent with the development of a euthyroid sick syndrome. Depression levels did not change from the first to the second interval although there was a small increase in psychosis rating indicating that the patients remained ill during this period of time and did not show spontaneous remissions. The changes in the thyroid indices were most pronounced in the patients who had been rapid cyclers (demonstrating more than four episodes per year in the year prior to NIMH admission) and in those who demonstrated hypercortisolism (increased excretion of urinary free cortisol) during their medication-free evaluation at NIMH. These data are of interest in relationship to reports that a variety of medical illnesses and glucocorticoid treatment can induce the euthyroid sick syndrome.

We observed a pattern of increased thyroid indices being positively correlated with measures reflecting increased rapidity of cycling and a greater severity of illness. Moreover, rapid cyclers had significantly higher levels of T_4 and free T_4 than nonrapid cycling patients. There was no relationship of TSH levels to degree of rapid cycling. Duration of time off lithium did not account for the findings and was unrelated to differences in thyroid indices. In fact, in a subgroup of patients exposed to lithium, the relationship of rapid cycling to higher levels of thyroid hormone remained.

Thus, a consistent perspective on the relationship of thyroid dysfunction to affective illness emerges in our data. Rapid cyclers are characterized by relative hyperthyroid, rather than hypothyroid, indices (although most values are within the normal range) and respond to drugs that tend to further reduce thyroid indices, such as lithium and carbamazepine (see above). Moreover, we observed that responders compared with nonresponders to the acute antimanic effects of carbamazepine showed significantly greater levels of T_4 and free T_4 in the period prior to carbamazepine treatment. These data are also consistent

with the findings of others that some 30-40% of affectively ill patients show blunted TSH responses to TRH and only a small minority show increased responses. These data from the literature are also consistent with relative thyroid hyperfunction rather than hypofunction. Moreover, recent data from Nemeroff et al indicate increased TRH levels in the CSF of depressed patients, and Reichlin and associates reported that chronic intrathecal TRH administration for patients with amyotrophic lateral sclerosis produces a profile of thyroid indices similar to that observed in depressed patients with normal to high hormone levels and a blunted response to TRH.

Joffe et al have suggested that this view of relative thyroid hyperfunction in affective illness may also be consistent with the data that antidepressant responses can be potentiated with supplemental thyroid hormone administration with T_3 . T_3 , by feedback inhibition, actually suppresses circulating levels of T_4 . Since CNS uptake is dependent on circulating levels of T_4 and intracellular conversion to T_3 , this thyroid manipulation may actually induce relative thyroid hypofunction, like many of the other treatments of affective illness. Joffe predicted, on the basis of this hypothesis, that depressed patients would respond better to T_3 potentiation than to T_4 ; preliminary data from his group in Toronto now support this prediction.

b. Suicidality and Course in Affective Illness: We have found that 49 of 87 of our affectively ill patients (56%) had made suicide attempts. Females (34/51 or 66%) were more likely than males (15/36 or 41%) to have made an attempt. While the majority of attempts occur within the first year or two of illness, more severe attempts (as assessed by a formal "risk" scale) were significantly correlated with duration of illness ($r = .52$, $p < .004$, age corrected) and total number of affective episodes ($r = .40$, $p < .03$). Intensity of suicidal ideation (which was higher in attempters than nonattempters) was not highly correlated with lethality of attempt, but was correlated with several variables including number of episodes of illness. This study provides one of the first attempts to examine suicidality in affectively ill patients as it relates to the longitudinal course of affective illness.

6. Depressive Subtypes and Symptoms in Relation to Regional Localization of Function

a. Psychosensory Phenomena - Comparison with Epileptics: In collaboration with Dr. P. Hauser, we have continued to assess signs and symptoms that are usually associated with psychomotor epilepsy in patients with primary affective illness and panic-anxiety illness, as well as in patients with temporal lobe epilepsy and in a medical control group of hypertensive patients. Compared with the medical control group, patients with affective illness, panic-anxiety disorders, and with epilepsy showed a highly significant increased incidence in the number of these signs and symptoms. The qualitative symptom profiles differ slightly among the affective, anxious, and epileptic patients. Depressed patients with a history of panic attacks have more symptoms than depressed or anxious patients without panic attacks and show a profile highly characteristic of panic patients. To the extent that psychosensory distortions and related symptoms usually associated with temporal lobe epilepsy are occurring with a high incidence in patients with primary affective illness, these data might sug-

gest that some of the neural substrates involved in complex partial seizures overlap with affective illness. Contrary to predictions, affective patients with greater numbers of psychosensory symptoms responded better to lithium carbonate, and there was no significant relationship for carbamazepine.

Drs. L. Altshuler and O. Devinsky have examined mood and anxiety in patients with complex partial seizures. Patients with a left-sided seizure focus showed higher levels of anxiety compared with those with focus on the right or compared with controls.

b. Psychological, Structural, Metabolic, and Electrophysiological Approaches to Regional Brain Function in Affective Illness: A variety of psychological test batteries are employed to assess possible alterations in regional brain function in patients with affective illness, including the Luria Battery and the Halstead Categories Test. Impairment in cognitive function has been documented on these tests during depression. Depressed patients compared with controls are also deficient in their ability to recognize emotions in pictures of faces presented to them; recognition of expressions of sad and elated are particularly disturbed (Rubinow et al).

Computerized axial tomography (CAT) scans have been performed on our patients with affective illness and reveal a similar range of ventricular brain ratios (VBRs) comparable to those observed in schizophrenic patients. Larger VBR is not associated with a more chronic or recurrent course of illness in manic-depressive patients, as has been reported in some studies of schizophrenics. Patients with cortisol hypersecretion and greater cognitive defects had larger VBRs. Brain structure can be examined in exquisite detail in patients using magnetic resonance imaging (MRI). Drs. P. Hauser and L. Altshuler have focused their investigations on temporal lobe structures in patients with manic-depressive illness. Compared with normal volunteer controls, patients with manic-depressive illness have a decreased temporal lobe area measurement and this is evident on both the left and right sides of the brain. The temporal lobe area is measured as a ratio to the rest of the brain area on a given MRI scan in order to control for positional and other artifacts. It was also observed that the left temporal lobe to cerebral ratio was significantly smaller than that on the right or nondominant hemisphere. This occurred in both patients and controls. This temporal lobe to cerebral ratio on the right significantly decreased with age in the normal volunteers but not in the affectively ill patients. This ratio was significantly correlated with duration of illness. These data are thus of interest from several perspectives. They suggest that the recent reports of similar alterations in temporal lobe structures in schizophrenia may not be specific to that diagnostic entity and raise the possibility that structural alterations in the temporal lobe could be relevant to the course of affective illness. The potential relationships between our preliminary findings of decreased temporal lobe ratio to the pathophysiology of affective disorder remains to be carefully delineated.

Positron emission tomography (PET) scan studies using 2-deoxyglucose indicate glucose utilization in temporal cortex relative to other areas in the same brain slice was also lower in depressed patients compared with controls (studied

with Drs. Cohen, DeLisi and Buchsbaum). These data provide evidence that depressed patients differ from patients with complex partial seizures who show areas of increased glucose utilization in the temporal lobe ictally and hypometabolism interictally (Engel et al, 1982).

c. Procaine Infusions as a Probe of Limbic System Responsivity:

Graded doses of the local anesthetic procaine were administered to affectively ill patients (in collaboration with Drs. C. Kellner, F. Putnam, and M. Kling), borderline personality disorders (in collaboration with R. Cowdry and D. Gardner), and normal volunteers in an attempt to probe limbic system responsivity. Analysis of the first 21 subjects by Dr. R. Coppola reveals selective increases in fast EEG activity, especially 26 to 45 Hz over the temporal cortex, confirming in man the suggestions from animal studies that local anesthetics activate temporal lobe and limbic structures. Dose-related alterations in subjective sensory and cognitive functions were reported as well as a variety of affective responses ranging from mood elevation to dysphoria. Vivid recall of experientially immediate memories, as well as hallucinatory-like phenomena, occurred less often. In patients with borderline personality disorder, degree of fast activation of the temporal cortex was not positively correlated with response to carbamazepine (Cowdry and Gardner). Procaine-induced release of ACTH, cortisol, and prolactin, but not growth hormone, has also been documented in collaboration with Drs. P. Gold, C. Kellner, and M. Kling. These data suggest the utility of procaine as a potential pharmacological probe of the limbic-temporal lobe function.

7. Laboratory Studies of Cocaine-Induced Behavioral Sensitization and Pharmacological Kindling (in collaboration with Drs. S.R.B. Weiss and Agu Pert)

Cocaine-related behavioral and physiological changes are studied in animals for several reasons. Cocaine induces complex effects on mood and behavior in man; initial manic-like euphoria may give way to mounting anxiety, dysphoria, paranoia, and panic attacks with increasing use. Thus, cocaine may model several of the major psychiatric illnesses and give hints to mechanisms underlying their evolution and pathophysiology. It may also be an interesting model "stressor", particularly since it both increases catecholamines and appears to release corticotropin releasing hormone (CRH). The effects of cocaine on CRH have recently been documented by Calogeras, Gold, and associates, as well as by Rivier and Vale. We have observed a dose frequency relationship of cocaine's effects on behavior. Small doses of cocaine did not lead to an increased amount of motor activity on rechallenge. However, many repetitions of low doses of cocaine do lead to this increased response (behavioral sensitization). Single high doses of cocaine (40 mg/kg i.p.) will also produce behavioral sensitization which is environmental-context dependent. With greater numbers of repetitions, the increased behavioral response becomes environmental-context independent; i.e., less dependent on conditioning factors. With sufficient repetitions, animals will further develop a pharmacological kindling and demonstrate seizures to this same 40 mg/kg dose which was previously subconvulsant. Psychomotor stimulant effects of cocaine are thought to mediate behavioral sensitization, while the local anesthetic properties of cocaine are related to its pharmacological kindling of seizures.

Finally, in addition to the use of cocaine as a model for psychiatric illness and as a pharmacological "stressor", its behavioral and physiological effects are currently of great interest in their own right because of the increasing prominence of cocaine use in the United States and the current cocaine epidemic. Many of the findings on behavioral sensitization and pharmacological kindling have important implications for behavioral and physiological toxicities to cocaine with chronic administration.

a. Conditioning in Cocaine-induced Behavioral Sensitization: We have investigated the phenomenology and mechanisms underlying the increased behavioral responsivity to the same dose of the psychomotor stimulant cocaine. Animals administered cocaine (10 mg/kg i.p.) once-daily show increasing amounts of locomotor hyperactivity and stereotypy to the same dose over time. An environmental context or conditioning component has been demonstrated. For example, animals repeatedly treated with cocaine in the same context (the test cage) showed greater degrees of hyperactivity and stereotypy than animals receiving identical doses in a different environment and then injected in the test cage. These findings have been replicated using drug or saline injections into the nucleus accumbens; cocaine pretreated animals showed an increased response to intracerebral saline or amphetamine only when they had been pretreated in the same environment. The similarity of the pretreatment environment (where and in what type of cage animals receive cocaine [40 mg/kg] on day 1) to the test environment where they receive cocaine (10 mg/kg) on day 2 is also related to the degree of sensitization.

The experience of motor hyperactivity itself in the pretreatment environment following cocaine challenge (40 mg/kg) appears necessary for cocaine-induced behavioral sensitization to occur to a cocaine (10 mg/kg) test dose the next day. If cocaine-induced activity during the pretreatment is blocked with haloperidol, diazepam, but not muscimol, sensitization to cocaine (10 mg/kg) does not occur. (Diazepam and muscimol pretreatments in themselves increase subsequent responsivity to cocaine, suggesting that GABA mechanisms may facilitate subsequent response to cocaine.)

b. Neuroleptics Block the Development, But Not Expression of Cocaine-induced Behavioral Sensitization -- A Model of Neuroleptic Non-responsiveness: It is also of interest that while neuroleptic blockade of cocaine-induced hyperactivity during the day 1 cocaine pretreatment phase blocks sensitization, neuroleptic administration prior to the day 2 testing phase does not block the sensitizing effect of cocaine. Two doses of haloperidol (0.2 mg/kg and 0.5 mg/kg) that were sufficient to block the development of sensitization when administered on day 1, were both unable to block the expression of sensitization when administered on day 2. To the extent that behavioral sensitization accounts for some of the progressive development of psychopathology to cocaine in man, these data suggest that neuroleptic treatment, once sensitization has already developed, will be ineffective. These findings may also represent an animal model for neuroleptic nonresponsiveness in some psychotic conditions.

c. Anatomical Substrates for Cocaine-induced Behavioral Sensitization: Using lesion strategies, we have attempted to dissect possible neural substrates mediating the conditioned component of cocaine-induced behavioral

sensitization. We found that selective dopaminergic lesions of the nucleus accumbens that were insufficient to block day 1 high-dose cocaine-induced hyperactivity did block the expression of cocaine-induced behavioral sensitization. Similarly, electrolytic lesions of the amygdala as well as selective dopaminergic lesions of the amygdala, blocked cocaine-induced behavioral sensitization. This effect was not achieved by lesions of the dorsal or ventral hippocampus or midline cerebellar structures. These data suggest that nucleus accumbens and amygdala and, in particular, the dopaminergic components of these pathways, may be involved in the mediation of cocaine-induced behavioral sensitization.

d. Cocaine-induced Pharmacological Kindling: Repeated, intermittent electrical stimulation of the brain results in increasing duration, spread, and complexity of electrical after-discharges culminating in the appearance of major motor seizures to a previously subthreshold stimulation (Goddard et al, 1969). Repeated daily injections of the same dose of lidocaine (65 mg/kg, i.p.) also lead to an increasing incidence, severity, and duration of seizures to the same dose over time, a phenomenon we have called pharmacological kindling. An equal local anesthetic dose of cocaine (65 mg/kg) also produces pharmacological kindling, but with a much higher seizure incidence and lethality.

e. Carbamazepine Inhibits the Development of Cocaine Kindling: Carbamazepine is a potent inhibitor of the development phase of lidocaine-kindled seizures, but is ineffective against completed lidocaine seizures. Chronic carbamazepine also very effectively inhibits the development of cocaine-kindled seizures and their accompanying lethality, but is ineffective in preventing high-dose cocaine-induced seizures and may even increase the cocaine-induced lethality when given acutely (S.R.B. Weiss). Carbamazepine does not block the development or expression of cocaine-induced behavioral sensitization in rats and does not block stimulant- (methylphenidate) induced euphoria in man. We have postulated that cocaine-induced panic attacks develop in a kindling-like fashion and are related to cocaine's local anesthetic effects (Uhde and Weiss). To the extent that this is the case, we would predict that carbamazepine would block the development of cocaine-related panic in man (local-anesthetic-related), but not block its ability to induce euphoria or paranoia (stimulant-related).

f. CRF Seizures and Behavior: Interaction with Amygdala Kindling: Dr. S.R.B. Weiss, in collaboration with Dr. A. Pert, has conducted a series of studies on the behavioral and convulsive effects of corticotropin releasing hormone (CRF) administered intracerebroventricularly. CRF induces the late onset (i.e., following a lag of approximately 4-8 hours post injection) of seizures that behaviorally and electrophysiologically resemble those produced from electrical stimulation of the amygdala. Following five repeated once-daily administrations, tolerance develops to the seizure inducing effects of CRF. Despite this, CRF seizures enhanced the development of amygdala-kindled seizures such that animals pretreated with CRF develop electrically kindled seizures twice as fast as vehicle-injected controls. CRF-treated animals also show increases in aggressive behavior toward other rats, an effect that was markedly enhanced in the electrically kindled rats. Conversely, electrically kindled rats showed a decreased convulsive response to CRF similar to that seen with repeated CRF injections.

The convulsive response to CRF was not reliably reproduced by local intracerebral injection into amygdala, hippocampus, septum, hypothalamus, periaqueductal gray (PAG) or the pre-pyriform area identified by K. Gale as a highly sensitive trigger zone for other seizures. However, the aggressive behavior could be elicited by CRF injections into PAG. Moreover, small lesions of the amygdala decreased the CRF-induced aggression following i.c.v. administration, but small or large amygdala lesions did not affect the development of seizures. Lesions of the hippocampus, pre-pyriform area, and olfactory tubercle similarly did not block the development of seizures produced by i.c.v. CRF.

These data suggest that CRF is inducing seizures behaviorally similar to those produced by electrical stimulation of the amygdala, but they are not dependent on an amygdala substrate for their occurrence. Further, these data suggest the possibility that an endogenously produced, stress-related peptide such as CRF may, under pathological conditions, be associated with alterations in convulsive and aggressive responsivity. No discrete brain focus of this effect has so far been found and since 50-100 μ g of CRF into the CSF appears to be required, this effect may be pharmacological rather than physiological.

D. Proposed Course of Project

We have helped to introduce and document carbamazepine as an effective treatment modality for manic-depressive and schizoaffective illness. Preliminary predictors of clinical response have been elucidated. We propose to further delineate clinical and biological markers of carbamazepine response. Preliminary evidence suggests that many patients who clearly do not respond to lithium carbonate will respond to carbamazepine. It will be increasingly important to establish whether response to carbamazepine, compared with lithium carbonate, delineates separate subgroups of patients with affective illness. As such, carbamazepine responders might be distinguished on the basis of: 1) severity; 2) pattern (rapid cycling); 3) genetics (negative family history); 4) course of illness (late); or 5) biological markers. Out-patient studies headed by Dr. K. Denicoff will address several of these issues in a controlled trial of lithium, carbamazepine, and the combination.

The degree of generalization of carbamazepine response to other anticonvulsant agents such as valproic acid, clonazepam, or phenytoin will be another area of both clinical and theoretical import. This is also particularly the case in light of our findings that electroconvulsive shock exerts potent anticonvulsant effects on limbic system seizures. Are anticonvulsant effects of a variety of treatment modalities (including ECT) linked to therapeutic response in affective illness? Carbamazepine is clearly useful in pain syndromes that do not involve a convulsive process, and effectiveness of anticonvulsant agents in a subgroup of patients with affective illness does not imply an underlying ictal process.

The possible mechanisms of action of carbamazepine in our patients, as well as in behavioral pharmacological models, will also be pursued. The clinical trial of baclofen will help elucidate the role of GABA-B mechanisms in carbamazepine's efficacy. We will investigate whether carbamazepine's anticonvulsant metabolite, carbamazepine-10,11-epoxide, also has important psychotropic properties in manic-depressive patients.

Alterations in somatostatin as they relate to affective and seizure mechanisms will also be systematically explored, especially in light of growing evidence of alterations in somatostatin in depression and in a variety of neuropsychiatric disorders (D.R. Rubinow).

As described in detail in Project #Z01 MH 00071-08 BP, Dr. T.W. Uhde will continue to explore the similarities and differences in patients with panic anxiety syndromes and those with affective illness in terms of acute symptomatology, longitudinal course of illness, and response to pharmacological agents. Catecholamines appear to be altered in both the mood disorders and in panic anxiety disorders. Response to treatments which act on catecholamine systems such as clonidine will be compared and contrasted in both patient populations. Since caffeine has been shown to increase plasma cortisol and induce escape from dexamethasone suppression, the clinical, mechanistic, and theoretical implications of this important observation will be systematically followed up by Dr. Uhde and his associates.

Dr. D.R. Rubinow is continuing to study and treat patients with menstrually-related exacerbation of mood and behavior disorders. He will be examining this problem from a clinical and endocrinological point of view, and as a model for studying the acute onset and offset of affective dysfunction.

Work in animal models will continue to focus on possible mechanisms underlying behavioral sensitization and electrophysiological kindling. In collaboration with Drs. S.R.B. Weiss, P. Marangos, J.-L. Daval and T. Nakajima, neurotransmitter receptors, protein phosphorylation, ion channels, and oncogenes such as C-fos will be examined as possible mediators or modulators of the electrophysiological kindling paradigm.

The mechanisms of anticonvulsant action of carbamazepine on amygdala-kindled seizures will also be further studied. We will attempt to elucidate the critical variables involved in the phenomena of conditioned-inefficacy and conditioned-tolerance to carbamazepine and optimize treatments to prevent their development. The role of environmental context and conditioning will also be examined in these paradigms; anatomical and biochemical substrates will be studied.

The mechanisms of cocaine-induced kindled seizure lethality will be explored. The fact that carbamazepine blocks the development of cocaine-induced kindled seizures and lethality will be further explored for its potential clinical utility and as a seizure model that requires chronic carbamazepine in order to be effective; thus, it may be a useful model for examining potential mechanisms underlying carbamazepine's efficacy in manic-depressive illness, where chronic drug administration is required in order for the antimanic and antidepressant effects of the drug to be observed.

E. Significance to Biomedical Research and the Program of the Institute

Based in part on work in this Branch, carbamazepine has emerged as a new treatment for manic-depressive illness. The anticonvulsants have emerged as a class of new treatments as valproic acid and clonazepam also appear effective in mania. Thus, a whole new range of treatment options has evolved from the work with carbamazepine.

Carbamazepine's clinical and theoretical importance is further highlighted by the fact that it is effective in some patients who do not respond to lithium carbonate. Studies of the mechanism of action of carbamazepine may provide new leads to the understanding of mechanisms of action of other effective antimanic and antidepressant drugs as well as basic mechanisms underlying affective dysregulation. Mechanisms can now be compared and contrasted with lithium and also with a range of other anticonvulsants that are effective in manic-depressive illness. Thus, basic and clinical research has led to important findings in neurobiology, the development of a new treatment for affective illness with carbamazepine, and prospects for more specific treatments in the future. NIMH has been recognized as the pioneer in this area of study.

Study of endocrine and peptide substances in man and animals may also provide new conceptual and practical treatment approaches to the relationship between manic and depressive symptoms and biochemistry. Examination of the interaction between classical neurotransmitters and the peptides should prove fruitful in understanding normal and pathological functioning. The multidisciplinary assessment of our patients' mood, behavior, cognition, physiology, and biochemistry should allow more precise characterization of important biobehavioral relationships and their underlying neural substrates.

Elucidating the mechanisms underlying behavioral sensitization and kindling, which appear to involve processes akin to memory, may provide important information regarding the coding of behaviorally relevant long-term changes in the CNS. Kindling and behavioral sensitization have aided in the conceptualization of a variety of psychiatric disorders that show progressive increases in behavioral pathology over time, and have led to the introduction of new treatment strategies as well as the novel conceptualization of the efficacy of pharmacotherapy as a function of state of syndrome development. This is clearly documented with neuroleptics, which block the development, but not expression, of cocaine-induced behavioral sensitization, and with carbamazepine, which blocks completed amygdala-kindled seizures, but not their development.

Studies of chronic cocaine indicate a potent conditioned component to behavioral sensitization. Conditioning is now also being recognized as a major factor in the clinical treatment of cocaine-addicted patients. If subjects are exposed to cocaine-related paraphernalia or similar cues even months after they have been withdrawn from the drug, powerful craving and/or withdrawal symptoms can be re-introduced. Thus, elucidation of the mechanisms and anatomical and biochemical pathways involved in conditioned components of cocaine-induced behavioral sensitization in the preclinical animal models should lead to a better understanding of related phenomena and possible new treatment alternatives in man.

Similarly, understanding the mechanisms involved in the high lethality of cocaine-induced kindled seizures may lead to better treatment interventions for this potentially catastrophic reaction. Both the behavioral sensitization and kindling perspective, to the extent that they can be extrapolated to the human cocaine use condition (and all of the data so far suggest that they can be), imply that there may be additional hidden liabilities to cocaine use beyond

those that are widely known. That is, that both behavioral and convulsant toxicity may become an increasing problem with repeated use and that a given dose of cocaine, which was previously well tolerated, upon sufficient repetition, may not only lead to increasing pathological effects on behavior, but, in some instances, may produce lethal seizures as observed in pharmacological kindling. Thus, these preclinical findings which are of interest in their own right and as potential models for manic and other psychotic symptomatology and psychiatric disorders, may also be of considerable public health interest and pave the way for reconceptualization of the pathophysiology of cocaine-related syndromes and new treatment interventions.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZOI MH 00071-08 BP
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychobiological Correlates and Treatment of Panic and Related Mood Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) T.W. Uhde, M.D., Chief, Unit on Anxiety and Affective Disorders, BPB, NIMH <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> R.M. Post, M.D. J.-P. Boulenger, M.D. P.P. Roy-Byrne, M.D. B.J. Vittone, M.D. B. Scupi, M.S.W. </div> <div style="width: 45%;"> BPB, NIMH French National Institute for Health and Medical Research, Cannes, France Univ. of Washington, Seattle, Washington BPB, NIMH BPB, NIMH </div> </div>		
COOPERATING UNITS (if any) BPB,CNB,CRB,LCS,LPP,NSB,NIMH; PND,NIMH; LCS,NIAAA; ETB,NINCDS; Fr. Nat'l Inst. of Hlth. & Med. Res.; UC, Irvine & SD; Bronx VA Med. Ctr.; Med. U. of SC; Univs. of Pittsburgh, Miami, Wash., Pa.; Mayo Clinic; Cath. U.; Beth Israel Hosp.; Nerv. der U., Munich; Israel I. of Tech.; Gensia Pharmaceuticals		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Anxiety and Affective Disorders		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">5</div>	PROFESSIONAL: <div style="text-align: center;">4</div>	OTHER: <div style="text-align: center;">1</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Patients with pathological degrees of <u>anxiety</u> who meet DSM III-R criteria for <u>panic disorder, with or without agoraphobia; generalized anxiety disorder; or social phobia</u> are evaluated using psychological, physiological, and biochemical methodologies. Patients with <u>major affective illness</u>, particularly those with a significant anxiety component, are also eligible for participation in the program. Particular attention is given to the role of the <u>noradrenergic, dopaminergic, adenosinergic, and serotonergic neurotransmitter systems</u> as assessed by: 1) measurement of the metabolites NE, MHPG, and HVA in plasma; 2) adrenergic receptor number and function in platelets; and 3) neuroendocrine and behavioral responses to the alpha-2 adrenergic agonist <u>clonidine</u> and antagonist <u>yohimbine</u> and the serotonin agonist <u>m-chlorophenylpiperazine (MCP)</u>. Research investigating the relationship of noradrenergic and adenosinergic function to other neurotransmitter systems and the <u>hypothalamic-pituitary-adrenal</u> and <u>hypothalamic-pituitary-thyroid axes</u> also has been initiated. Caffeine and nifedipine challenges are administered to assess their behavioral and biochemical effects. Other approaches to understanding the pathophysiology of anxiety and its potential treatment with <u>alprazolam, carbamazepine, clonidine, dipyridamole, imipramine</u> and <u>verapamil</u> will be explored. </p> <p> An animal model using genetically "nervous" and "normal" pointer dogs has been developed and studied in relation to noradrenergic and adenosinergic function. </p>		

COLLABORATORS:

M. Geraci	Psychiatric Nursing Department, NIMH
M.S. Buchsbaum, M.D.	University of California at Irvine
T.P. Zahn, Ph.D.	LPP, NIMH
M. Kafka, Ph.D.	NSB, NIMH
L. Siever, M.D.	VA Medical Center, Bronx, New York
D.C. Jimerson, M.D.	Beth Israel Hospital, Boston, Massachusetts
N. Salem, M.D.	LCS, NIAAA
M. Albus, M.D.	Nervenlinik der Universitat, Munich, W. Germany
W. Potter, M.D.	LCS, NIMH
M. Linnoila, M.D.	LCS, NIAAA
L. Bierer, M.D.	VA Medical Center, Bronx, New York
C. Kellner, M.D.	Medical University South Carolina, Charleston, South Carolina
E. Klein, M.D.	Israel Institute of Technology, Haifa, Israel
J. Maser, Ph.D.	CRB, NIMH
T. Mellman, M.D.	BPB, NIMH
G. Leverich	BPB, NIMH
K. Kramlinger, M.D.	Mayo Clinic, Rochester, Minnesota
P. Marangos, Ph.D.	Gensia Pharmaceuticals, San Diego, California
S. Risch	Univ. of California at San Diego
S. Sinclair	University of Miami, Miami, Florida
W. Kaye, M.D.	Univ. of Pittsburgh, Pittsburgh, Pennsylvania
S. Steinberg	Veterinary Medicine, Univ. of Pennsylvania
S.R.B. Weiss, Ph.D.	BPB, NIMH
M. Stein, M.D.	BPB, NIMH
G. Gurguis, M.D.	BPB, NIMH
C. Shea, M.A.	BPB, NIMH
D. Arnkoff, Ph.D.	Catholic University, Washington, D.C.
I. Heuser, M.D.	ETB, NINCDS
J. Juncos, M.D.	ETB, NINCDS
M. Tancer, M.D.	BPB, NIMH
W. Berrettini, M.D.	CNB, NIMH

I. Project Description

A. Objectives

This project employs a multidisciplinary team in the study and treatment of pathological anxiety, major affective and related mood disorders.

B. Methods Employed

1. Subjects

a. Patients who meet Research Diagnostic Criteria for panic, phobic, generalized anxiety disorders, and social phobia, as well as patients who meet DSM III criteria for major affective illness, are candidates for participation in the project. Inpatients are studied and treated on the 3-West Clinical Research Unit and outpatients are followed through the Ambulatory Care Research Facility. A number of previously validated scales to measure state and trait anxiety are utilized, and an analogue anxiety scale and panic anxiety scale have been developed to more adequately assess the relationship among state anxiety, phobic anxiety, avoidance behavior, and depressive symptomatology.

b. Normal volunteers are also accepted into the project to provide control data, as well as to assess the relationship between normal state anxiety and selected psychological and biological variables.

2. Psychological and Biological Evaluation

a. Baseline Evaluation. During an initial evaluative period patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation. This initial evaluation is indicated due to the heterogeneous nature of the panic and phobic disorders. Anecdotal reports suggest that many medical illnesses may present as or exacerbate pre-existing conditions of pathological anxiety.

b. Life Chart Methodology. A life chart technique has been developed to plot the frequency, intensity, and interval between panic attacks. The character and the change in the quality of panic attacks is assessed as a function of duration and longitudinal course of illness. This approach allows the Unit to document the development, recurrence, and progression of the panic and phobic disorders. Life charting is an important aspect of the overall project because few systematic studies have been conducted on the natural progression of these disorders.

As part of this assessment, life events and their impact on the course of illness are investigated with the PERI-M life events inventory. Moreover, the influence of personality (DSM III, Axis II diagnosis) on the phenomenology and course of illness is systematically evaluated with the Structured Interview for DSM III Personality Disorders (SIDP). These studies are conducted in collaboration with Drs. K. Kramlinger, T. Mellman, and with M. Geraci, G. Leverich, and B. Scupi.

c. Sleep and Sleep Deprivation. Electroencephalographic sleep recordings are obtained for three consecutive nights. Although many panic anxious patients, like endogenously depressed individuals, have improved sleep following treatment with tricyclic and monoamine oxidase inhibitors, little is known about the sleep architecture of panic and phobic anxious patients. The physiological correlates of sleep panic attacks represent a major focus of the unit's sleep EEG studies. The effects of one night's sleep deprivation on mood and behavior are also investigated in patients with panic disorder and major depressive disorder. Sleep studies are conducted in collaboration with Drs. T. Mellman and P. Roy-Byrne.

d. Galvanic Skin Response. The effects of yohimbine on physiological measures of galvanic skin response, reaction time to auditory tones, pulse, and respiratory rate are studied in panic and phobic anxious patients and age-matched normal volunteers. This investigation is performed in collaboration with Drs. M. Albus, B. Vittone, and T. Zahn.

e. Computerized Axial Tomography. Cerebral CAT Scans are obtained, and, in collaboration with Dr. C. Kellner, cerebral ventricular size is determined in patients with panic disorder. Scans are performed on a GE 8800 or 9800 Scanner.

f. Psychomotor Activity. Twenty-four hour motor activity is assessed with a miniaturized activity monitor worn on the wrist of patients with primary anxiety disorders under a variety of experimental conditions.

g. Caffeine. Caffeine is administered to panic patients and normal controls to assess behavioral and biochemical responses to this agent whose effects are thought to be mediated through the adenosine, GABA-benzodiazepine, and noradrenergic systems.

To assess the effects of caffeine on plasma adenosine, an HPLC assay for adenosine and caffeine has been developed in collaboration with Drs. N. Salem and P. Marangos. The clinical studies are conducted in collaboration with Drs. L. Bierer, J.-P. Boulenger, T. Mellman, R. Post, M. Stein, and M. Geraci and S. Sinclair.

h. Clonidine -- An Alpha-Adrenergic Agonist. Clonidine is administered intravenously to panic disorder, social phobic, and affectively ill patients and normal volunteers to assess clinical, physiological, and neuroendocrine responses to this noradrenergic drug. These studies are conducted in collaboration with Drs. M. Tancer, G. Gurguis, W. Kaye, R. Post, L. Siever, and B. Vittone.

i. Yohimbine -- An Alpha-Adrenergic Antagonist. Yohimbine is administered in an oral challenge to panic anxious and affectively ill patients and normal controls to assess the clinical and biochemical effects of this noradrenergic antagonist which is known to potentially increase noradrenergic function in the animal. These studies are conducted in collaboration with Drs. M. Albus, G. Gurguis, B. Vittone, T. Zahn, and with M. Geraci.

j. GRF Stimulation Test. Growth hormone releasing factor (GRF) is administered intravenously to patients with panic disorder, social phobia, affective disorders, and age- and sex-matched normal controls in order to assess the

physiologic, behavioral, and neuroendocrine responses to this hypothalamic peptide. Collaborators include Drs. M. Tancer, M. Stein, and G. Gurguis.

k. TRH Stimulation Test. TRH is administered intravenously to patients and age- and sex-matched normal controls to evaluate the hypothalamic-pituitary-thyroid axis.

l. Plasma MHPG, HVA, and Urinary Free Cortisol. Amine metabolites and urinary free cortisol are systematically evaluated in panic disorder and social phobic patients using daily 24-hour urine collections across clinical state changes on and off medication. These studies are conducted in collaboration with Drs. G. Gurguis, D. Jimerson, M. Linnoila, M. Tancer, W. Potter, and C. Shea.

m. Dexamethasone Suppression Test. Dexamethasone is administered to panic disorder and social phobic patients to evaluate the hypothalamic-pituitary-adrenal axis. Basal values are performed at baseline and at 4:00 pm, following dexamethasone administration.

n. Urine and Plasma Studies. Amine metabolites, electrolytes, and peptides are also measured in the urine and blood.

o. Alpha-Adrenergic Receptors. In collaboration with Dr. M. Kafka, platelet alpha receptor function as well as prostaglandin-stimulated increases in cyclic-AMP are assessed in panic disorder patients and age-matched normal volunteers. Dr. G. Gurguis assesses alpha- and beta-adrenergic binding function in panic disorder patients.

p. Platelet Imipramine Binding. In collaboration with Drs. W. Berrettini and G. Gurguis, [³H]imipramine binding to platelets is measured in patients and normal controls.

q. 24-Hour Ambulatory Blood Pressure. Mean 24 hour BP profiles and BP and P changes during panic attacks are evaluated in panic disorder patients and normal controls.

r. Postural Challenge. Amine metabolites and catecholamines are measured in patients and age- and sex-matched normal controls as an index of sympathoadrenal activity. These studies are conducted in collaboration with Drs. M. Stein and G. Gurguis.

3. Treatment

a. Psychotherapeutic. Treatment and evaluation are conducted in individual and/or group supportive sessions. In addition, ongoing clinical case conferences are utilized. Collaborators in these studies include Drs. E. Klein, T. Mellman, B. Vittone, G. Gurguis, M. Stein, M. Tancer, C. Shea, and M. Geraci and B. Scupi.

b. Routine Somatic Treatment. Both routine and experimental compounds are evaluated during double-blind clinical trials. Ongoing clinical trials include calcium channel blockers, tricyclic antidepressants, monoamine oxidase in-

hibitors, dipyridamole, and minor tranquilizers. These studies are performed in collaboration with Drs. E. Klein, T. Mellman, B. Vittone, M. Stein, M. Tancer, G. Gurguis, and M. Geraci and B. Scupi.

C. Major Findings (Studies in Humans):

1. Co-morbid Medical Illnesses and Panic Disorder

Detailed physical, neuropsychiatric, and laboratory evaluations continue to be performed in patients admitted to our program. In the first four years of our program, approximately 60% of our panic patients had previously undiagnosed medical illnesses. A lower percentage of patients referred to our program during the past two years are now found to have concomitant medical diseases. The previous high percentage of undiagnosed illnesses very probably represented an artifact of primary care physicians' attributing all somatic symptoms as a manifestation of the anxiety disorder. With increasing education regarding the manifestations and differential diagnosis of panic attacks, a greater percentage of patients appear to be appropriately evaluated prior to entry into the NIMH system. Nonetheless, we continue to be interested and actively investigate the role of psychological (major life events) and physiological (medical illnesses) stressors in triggering panic attacks in biologically vulnerable individuals. Moreover, we are interested in brain structure variables and co-morbid medical and psychiatric illnesses associated with panic disorder and other anxiety disorders.

a. Ventricular Brain Ratio. We continue to investigate the mean VBR in panic disorder patients. As reported in the Journal of Affective Disorders (1987), the ventricular brain ratios in our panic disorder patients fall well within the normal range compared with reported values of mean VBR in normal control groups in the literature. Of interest, however, we found a significant association between VBR and duration of benzodiazepine use ($r = 0.51$, $p < 0.02$) and percent of time ill treated with benzodiazepines ($r = 0.67$, $p < 0.001$), although the mean VBR (3.8 ± 2.5) of the panic disorder patients who had received benzodiazepine treatment was similar to the patients without previous benzodiazepine exposure (2.5 ± 1.6 ; $t = 1.26$, $df = 23$, $p = NS$).

The nature of the relationship between VBR and duration of benzodiazepine exposure ($r = 0.51$, $p < 0.02$) in our study remains unclear but might be related either to a direct or indirect drug effect or merely be an artifact reflecting a tendency toward greater drug use in a subpopulation of patients with more severe illness. Nonetheless, our findings underscore the importance of investigating whether benzodiazepine treatment over extended periods of time might be associated with changes in brain structure.

b. Complex Partial Seizures and Panic Disorder. Several investigators have suggested that some panic disorder patients may suffer from a variant of complex partial seizures, although no objective evidence has confirmed this hypothesis. Since psychosensory phenomena occur spontaneously and after stimulation of the amygdala and hippocampus in patients with psychomotor epilepsy, we continue to investigate the role of psychosensory symptoms in the phenomenology and longitudinal course of panic disorder. As noted in Z01 MH 00071-07 BP, panic disorder patients experience increased psychosensory symptoms during episodes of ill-

ness. During well intervals, the number of psychosensory symptoms in panic patients is similar to normal controls, although both nondepressed panic disorder patients and affectively ill patients report comparable increases in total number of psychosensory symptoms during clinical relapses. While these findings support an involvement of temporo-limbic structures in these psychiatric conditions, they do not prove the existence of electrical discharges in deep limbic structures. Moreover, our Unit has failed to find a relationship between the number and type of psychosensory symptoms and EEG activity with standard leads. Moreover, neither the frequency of psychosensory symptoms nor EEG variables have been found to predict the clinical response to a wide variety of psychotropic agents, including carbamazepine, in the treatment of panic disorder. The lack of relation between EEG measures and response to carbamazepine, a tricyclic anticonvulsant with a structure similar to the antipanic agent imipramine, is confounded by the lack of efficacy of carbamazepine in the treatment of EEG-normal panic disorder patients.

c. Thyroid Disease and Panic Disorder. A role for thyroid disease in the onset and maintenance of both panic and major depressive disorders has long been an area of interest in biological psychiatry. Our studies of peripheral thyroid indices in patients with panic disorder have failed to find significant abnormalities in comparison with normal controls. However, we had previously reported that patients with panic disorder tend to exhibit an increased incidence of subnormal thyrotropin (TSH) responses to thyrotropin-releasing hormone (TRH), a finding consistently reported in approximately 25% of depressed patients.

Consistent with our ongoing efforts to investigate the neurobiologic overlap between panic disorder and major depression, our Unit further evaluated potential etiopathologic similarities and differences in thyroid function between the two disorders. In addition to abnormalities in TSH response to TRH seen in both disorders, some patients with depression also exhibit the presence of antithyroid antibodies. The presence of antithyroid antibodies is suggestive of a diagnosis of "painless" or "silent" thyroiditis, a common thyroid disorder of presumed autoimmune origin. No studies to date had systematically examined the prevalence of antithyroid antibodies in patients with panic disorder, and to further clarify the relationship between panic disorder and major depressive illness, we conducted a study of antithyroid antibodies in 38 patients with panic disorder and 38 age- and sex-matched normal controls. Sex-matching is particularly indicated, since autoimmune thyroiditis is more likely to affect women.

Four patients with panic disorder (10.5%) and two healthy controls (5.3%) exhibited the presence of antithyroid antibodies (Fisher's Exact Test, one-tailed, $p = 0.34$). All six subjects with antithyroid antibodies were women, meaning that four of 27 women with panic disorder (14.8%) and two of 27 healthy women (7.4%) had antithyroid antibodies (Fisher's Exact Test, one-tailed, $p = 0.33$). Two women with panic disorder who had antithyroid antibodies continued under our care, thereby enabling us to document their titers serially over time. In both cases, their titers of antithyroid antibodies became undetectable within six months, and this time course did not closely parallel the course of illness of their anxiety disorder.

While this study indicates that active autoimmune thyroiditis is not an intrinsic biological substrate of panic disorder, there remain some intriguing

possibilities regarding the exact relation between some panic attacks and autoimmune thyroid dysfunction. Our observations that antithyroid antibody titers may diminish and eventually become undetectable over time demonstrates that our study is merely a cross-sectional assessment of these titers at a given point in time. Conceivably, the onset of the painless hyperthyroid phase of autoimmune thyroiditis could initiate or exacerbate panic attacks in susceptible individuals, with the eventual disappearance of antithyroid antibodies in some cases making detection and diagnosis at a later time impossible. It should also be noted that autoimmune thyroiditis is frequently seen to wane during pregnancy and then wax in the postpartum period. Of interest, this course parallels the pattern of many women with panic disorder who experience remission of their symptoms during pregnancy and recurrence in the postpartum period. It remains to be seen whether or not autoimmune thyroiditis plays a role in this pregnancy-related fluctuation in symptoms often seen in panic disorder, but this particular hypothesis certainly deserves further testing in longitudinal studies.

d. Parkinson's Disease and Anxiety Disorders. In order to study the prevalence and importance of anxiety disorders in patients with idiopathic Parkinson's disease, we systematically evaluated 24 parkinsonian patients for the presence of DSM-III-R-Axis I syndromes. Nine of 24 subjects (38%) had a clinically significant anxiety disorder. The severity of anxiety did not correlate with the degree of parkinsonian severity, the cumulative duration of L-dopa exposure, nor the current dose of L-dopa. These findings suggest that anxiety disorders may commonly afflict patients with Parkinson's disease, and should be considered in the medical evaluation and treatment of parkinsonian patients. In addition, our observations suggest that further attention should be paid to the role of the dopaminergic system in the etiopathology of anxiety and phobic disorders.

2. Co-morbid Psychiatric Conditions

There has been much recent interest in our unit in the investigation of panic disorder (PD) in relation to co-morbid risk for other psychiatric conditions, particularly affective and other Axis I anxiety disorders. Increasing evidence suggests that identifying subtypes of panic disorder in terms of overlapping symptomatology may have both important practical and theoretical implications. The following areas represent ongoing endeavors undertaken during the past two years.

a. Panic Disorder: Relation to Obsessive-Compulsive Symptoms. In our series of studies using validated diagnostic interviews, 27% of patients with panic disorder report obsessive-compulsive symptoms (OCS). In general, 53% report obsessional symptoms only, 10% report compulsive symptoms only, and 37% report obsessional plus compulsive symptoms.

Compared with panic disorder patients without obsessive-compulsive features, a lifetime history of major depression by DSM-III criteria is more frequent in the PD + OCS group ($p < .001$) as is a past history of alcohol or drug abuse ($p < .05$). The PD + OCS group patients has a significantly earlier onset of illness (20.3 ± 4.7 years) compared with patients without OCS (27.1 ± 7.7 ; $X^2 = 3.43$, $p < .01$). The two groups do not differ significantly with regard to the presence or severity of agoraphobia. There is no difference noted in the two groups for frequency of

panic attacks, total years ill, or percentage of time in remission prior to the NIMH evaluation.

There is also a significantly greater incidence of primary affective disorders (63% vs. 20%) and alcoholism or substance abuse (47% vs. 8%) in the first degree relatives of panic patients with OCS compared with the first degree relatives of patients without OCS. There is no difference in the incidence of panic or phobic disorders. While both groups improve with pharmacotherapy, persistent attacks are more common after treatment at follow up in the PD patients with OCS.

These data suggest that the presence of OCS in PD may be a clinical predictor of a subgroup of PD patients with distinct features and substantially less optimal treatment outcome, particularly in relation to symptom interference with functioning. The greater impairment in this subgroup cannot be attributed solely to the additional problems associated with a second separate neuropsychiatric disorder (i.e., obsessive-compulsive disorder), since these patients also reported a greater rate of panic attacks and more severe generalized anxiety at follow up.

b. Panic Disorder: Relation to Social Phobia. We studied the incidence of social phobic symptomatology and past history of major depression in 35 patients carrying a DSM-III-R diagnosis of panic disorder. Sixteen of the 35 patients (46%) received a DSM-III-R diagnosis of social phobia in addition to panic disorder, and of these 16, 15 (94%) reported past episodes of major depression. Only nine of the 19 panic disorder patients without social phobia (47%) had past histories of depression. When the panic disorder patients were subdivided according to affective disorder history, it was found that patients with a history of depression had significantly higher self-report questionnaire ratings of social anxiety and avoidance, but not of agoraphobic fear and avoidance, than the group without a history of depression. Our findings suggest that panic disorder and social phobia coexist in a substantial number of cases and that the presence of social phobia may be associated with significantly increased morbid risk for major depression in this population.

3. Life Events and Panic Disorder

During the past three years, the Unit has focused on the role of life stressors and life events in the onset and course of illness of panic disorder. Results of these studies indicate that patients with panic disorder, prior to the onset of their illness, personally experience more life events "happening to them" than controls. More importantly, life events seem to have a more adverse subjective effect on patients. In addition to comparing the frequency of life events in panic disorder patients versus normal controls, we also performed a within-patient analysis, comparing panic disorder patients who "had" versus "had not" experienced a major loss prior to the onset of their illness. There were no significant differences between groups in number of panic attacks, longest time free of panic attacks, or rapidity of onset of agoraphobia (45% within three months in the severe-loss group vs. 36% in the no-loss group). For the majority of patients suffering a subsequent depression, these depressions were severe and produced functional impairments regardless of whether or not there had been a severe loss pre-

ceding the onset of the illness. The time from first panic attacks to the depression and the total number of lifetime depressions were similar, whether or not there had been a prior loss.

These data suggest that the occurrence of major loss in patients with panic disorder confers an increased risk for a "secondary" depression without influencing the course of the primary anxiety symptomatology.

The major difficulty with this type of retrospective analysis involves the possible distorting effect of time on recall. Our analysis failed, however, to find an interaction between recall time and subject group by ANOVA. Using linear statistical analyses, the effects of time on recall was assessed in panic disorder patients and normal controls.

Not unexpectedly, time affected the number of events reported. However, there was no difference, in terms of the influence of time on the recall of each type of event, between the groups.

4. Sleep, Sleep-Panic, and Sleep Deprivation

Since a major focus of the Unit's ongoing research is the investigation of the relation between panic and major depressive disorders, we were particularly interested in the nature of rapid-eye-movement (REM) parameters in patients with panic disorder compared with normal controls. Preliminary data from our laboratory, published in *Psychiatry Research*, suggested that panic disorder patients did not have marked reductions in REM latency typical of patients with melancholic depression. In fact, in this initial study, our panic disorder patients had a significantly lower REM density and a normal progression in the length of each successive REM period. These findings have been both confirmed and extended by our laboratory in a second separate study.

In our second study, we investigated the sleep EEG of patients with panic and major depressive disorders and normal controls. The sleep of the panic disorder patients was generally disturbed, as manifested by significant decreases in sleep time and sleep efficiency and increased sleep latency. These disturbances were more prominent in the panic disorder patients compared with both the depressed patients and normal controls. Preliminary findings also suggest that REM latencies are reduced in depressed patients compared with the panic disorder patients and normal controls.

Six of 13 patients experienced sleep panic attacks. The sleep panics were all characterized as sudden awakening with fear or apprehension, without recall of any specific dream content. The symptoms of sleep panic attacks were generally typical of each patient's daytime panic attacks. The attacks occurred between 24 and 225 minutes from sleep onset and between 65 minutes before and 48 minutes after the first REM period. The epoch preceding the awakenings with panic were scored as stage 2 or early stage 3 sleep. No sleep panic attack was recorded during REM sleep.

That panic can occur in association with the progression toward a "deeper" stage of sleep is of interest with regard to the observation that increased basal arousal is often predictive of subsequent panic in many panic induction studies, such as those utilizing sodium lactate infusions. Our findings suggest that increased basal arousal is not a requirement for panic and that panic may actually occur in the context of diminishing arousal.

In our third separate study, we essentially confirmed indirect evidence from the literature and our own studies suggesting that EEG variables can be used to differentiate patients with major depressive disorder versus panic disorder versus normal controls. For example, new data collected in 22 panic disorder patients, 11 patients with major depressive disorder, and 9 normal controls revealed decreased sleep time in both patient groups compared with normal controls, but depressed patients had significantly less REM latencies compared with panic disorder patients.

5. Urinary Free Cortisol and Plasma MHPG in Panic Disorder

Alterations in noradrenergic function have been postulated to play an important role in the modulation of fear and anxiety. Moreover, in some psychiatric conditions, the noradrenergic system appears to be functionally related to hypothalamic-pituitary-adrenal (HPA) axis function. The Unit on Anxiety and Affective Disorders, therefore, studied urinary free cortisol and plasma MHPG in panic disorder patients and normal controls. Overall, our ongoing investigations suggest that there are no significant differences in either MUFC or plasma MHPG levels between panic patients and normal controls. Moreover, our Unit has failed to find any significant correlations between MHPG and frequency of panic attacks or measures of state anxiety, global anxiety, agoraphobia, or depression in the patients. There is also no significant correlation between MUFC and frequency of panic attacks or measures of state anxiety, global anxiety, agoraphobia, or depression in the panic disorder patients.

Our MUFC data suggest that increased HPA axis function is not a prominent feature of panic disorder. Our Unit has reported, however, that patients with panic disorder have elevated evening plasma cortisol levels and reduced ACTH and cortisol responses to corticotropin releasing hormone (CRH). Similar findings with the CRH test have been reported in depressed patients. These observations suggest that while panic patients may not have persistently elevated indices of increased HPA function, there may be discrete periods of hypercortisolemia associated with the illness. Mean plasma MHPG values did not differ between panic patients and controls.

We also failed to find a relationship between MHPG and frequency of panic attacks or ratings of global anxiety or agoraphobia. These observations also suggest that noradrenergic overactivity is not a biological prerequisite for all panic attacks or other types of pathological anxiety. Moreover, although an association between adrenergic activation and plasma cortisol have been reported in depressed patients, we found no correlation between plasma MHPG and urinary free cortisol in either panic disorder patients or normal controls. These observations, published in Biological Psychiatry, further suggest that the mechanisms underlying

central adrenergic and peripheral HPA activation may have different dimensions of functional relatedness, depending on the nature and state of the psychiatric syndrome.

6. Dopaminergic Function in Panic Disorder

To assess dopamine function in panic disorder, a series of ongoing studies have investigated plasma HVA in panic disorder patients and normal controls. In our first study, we found no difference in plasma HVA between panic disorder patients and normal controls. However, plasma HVA values showed a bimodal distribution. Comparisons of high and low HVA subgroups were tabulated. Patients in the "high" HVA subgroup, compared with those in the "low" subgroup, had significantly higher Spielberger State Anxiety scores, more panic attacks in the previous year, and shorter maximal time free of panic attacks.

Although this initial study suggested that panic disorder patients with higher concentrations of plasma HVA were more anxious, we have been unable to replicate these findings in a second, separate study. In this latter study, 22 patients and 12 normal controls gave informed consent to participate in the collection of 2-4 consecutive blood samples at 8-10 AM, after at least a 20-minute period of rest. The panic disorder patients had lower HVA levels (47.1 vs. 61.1, $p < .01$). Cortisol correlated negatively with HVA in patients ($r = -.34$, $p < .05$) but not in controls. These latter findings parallel similar observations in our animal model of anxiety in nervous pointer dogs and may offer as well a partial explanation for the observation that many panic disorder patients worsen following the administration of neuroleptic medications.

7. Effects of Diazepam on Cortisol and Beta-endorphin

Benzodiazepines have been shown to have neuroendocrine effects in both animals and humans. The most consistently observed endocrine changes in response to benzodiazepines have been decreases in ACTH and cortisol. Increases in growth hormone (GH) have been observed by some but not all investigators. Numerous studies have demonstrated that various kinds of stress can activate the HPA axis, causing increases in both cortisol and ACTH. Benzodiazepines have also been shown to antagonize stress-induced increases in both ACTH and cortisol in animals and humans.

We investigated, therefore, the neuroendocrine effects of diazepam in normal subjects under baseline (pre-stress) and laboratory-controlled stressful conditions. Although 10 mg doses of diazepam had no effect on cortisol at baseline, it significantly reduced the increase in cortisol produced by exposure to painful electrical stimuli. There were no significant effects on growth hormone, beta-endorphin, or ACTH either at baseline or following painful stimulation.

These results, to be published in the Journal of Clinical Psychopharmacology, document the ability of diazepam to blunt stress-induced increases in plasma cortisol in normal subjects. These results suggest the possibility of employing the cortisol-response to diazepam in assessing the function of the benzodiazepine system in different psychiatric conditions.

8. Caffeine: Behavioral and Biochemical Effects

Several ongoing studies are being conducted by the Unit on Anxiety and Affective Disorders to investigate the behavioral and biochemical effects of caffeine in panic disorder patients and normal controls. The following section reflects the scientific rationale and results of our research with caffeine.

a. Caffeine: Retrospective Survey. A caffeine-consumption survey was designed and administered to patients with panic and major depressive disorders and compared with normal controls matched for age, sex, and socioeconomic status. Data from this survey, published in Psychopharmacology Bulletin and the Archives of General Psychiatry, indicated an increased sensitivity to the psychostimulant and anxiogenic effects of caffeine in panic disorder patients compared with their normal controls. This relationship was not found in patients with major affective disorders. The findings of this survey, suggesting an increased vulnerability to the anxiogenic effects of caffeine in patients with panic disorder, led us to directly test the single-dose behavioral and biochemical effects of caffeine in panic disorder patients and normal controls. To pursue this goal, our Unit first investigated the effects of three separate doses of caffeine in normal controls.

b. Caffeine: Effects on Anxiety, Blood Pressure, Lactate, and Cortisol in Normal Controls. Using double-blind, placebo-controlled conditions, three doses of oral caffeine (240, 480, and 720 mg) were administered to 14 normal controls. Caffeine produced dose-related increases in state anxiety, mean arterial pressure, plasma lactate, and plasma cortisol. Plasma NE and its principal metabolite, MHPG, failed to increase. This research demonstrated that caffeine in sufficient doses may induce anxiety in normal subjects. The inconsistent effects of caffeine on MHPG suggest that noradrenergic systems might not be responsible for the major psychostimulant effects of caffeine in euthymic humans.

c. Caffeine: Effects on Plasma Adenosine Levels. The measurement of plasma adenosine after oral caffeine in humans was investigated because caffeine-induced behavioral changes in animals are thought to be mediated by blockade of adenosine receptors. In a group of eight normal volunteers, three oral doses of caffeine (240, 480, and 720 mg) and placebo were administered on four separate occasions. Despite dose-related increases in anxiety and plasma caffeine levels (up to 73.3 μM), no significant change in plasma adenosine concentrations was observed after caffeine administration. Although plasma adenosine levels did not change, these data support a role for adenosine receptor systems in caffeine-induced anxiety states since the caffeine levels reached after administration of 720 mg, the only dose which in this small sample produced significant anxiogenesis, are in a range (44-73 μM) known to compete with the binding of various ligands to the adenosine receptors in human brain (Ki-35-115 μM).

d. Increased Sensitivity to Caffeine in Panic Disorder Patients. To directly test our hypothesis that panic patients have an increased vulnerability to the anxiogenic effects of caffeine, a caffeine dose (480 mg) which failed to elicit severe degrees of generalized anxiety in normal controls, was administered under double-blind, placebo-controlled conditions to 24 panic disorder patients and compared with the 14 normal controls. The results of this study support our

hypothesis of increased sensitivity to caffeine in panic patients, as indicated by a significantly greater increase in measures of anxiety on the Zung Anxiety Scale in the patients compared with normal controls. Moreover, nine of 24 panic patients, but none of the 14 normal controls, experienced panic attacks by DSM III criteria. Compared with normal controls, the panic patients also had significantly higher levels of cortisol, lactate, and glucose following caffeine, although only increased levels of lactate distinguished between panicking and nonpanicking patients.

e. Caffeine Tolerance Test. In order to evaluate the effects of repeated caffeine challenge on mechanism of habituation or tolerance, caffeine (5 mg/kg) was given orally on eight consecutive days to ten panic disorder patients and eight normal controls.

All eight controls completed the study, but four of ten patients dropped out due to intolerable anxiogenic effects. These four dropouts had significantly higher anxiety ratings after the first caffeine challenge compared with the six patients who participated in all eight caffeine challenges. Of those subjects who completed the study, the patients demonstrated a delayed and, in some cases, an incomplete tolerance to the anxiogenic effects of repeated caffeine administration. The biochemical and physiological correlates of this delayed habituation are now under investigation and may lead to an improved understanding of the neurobiological mechanisms which mediate the regulation of anxiety.

f. Alprazolam Blocks Anxiogenic Effects of Caffeine. We have conducted preliminary studies investigating the effects of alprazolam, a triazolobenzodiazepine with antipanic properties in humans, on caffeine-induced anxiety. Blinded caffeine 480 mg was administered to patients participating in a double-blind, alprazolam-placebo crossover study. While six of 16 (37.5%) patients on the placebo phase of the study had panic attacks following single dose caffeine (480 mg), none of 11 (0%) of the alprazolam-treated patients had panic attacks following this same acute oral dose of caffeine ($p = .027$, Fisher's exact test). Of interest, alprazolam blocked the usual caffeine-induced increment in lactate and plasma cortisol levels. These behavioral and biochemical effects suggest that the benzodiazepine receptor system may play an important role in blocking some of caffeine's psychostimulant and biochemical effects. The role of the GABA-benzodiazepine receptor system in mediating caffeine's principal panicogenic effects remains to be elucidated.

g. Imipramine's Effects on Caffeine-induced Anxiety. We have conducted preliminary studies investigating the effects of chronic imipramine treatment on caffeine-induced anxiety. Our preliminary observations in this ongoing study suggest that imipramine is incapable of completely blunting the anxiogenic effects of caffeine. While a subset of subjects reported a change in their experience in response to caffeine while on imipramine (compared with previous challenges with caffeine while on placebo), the severity and character of responses varied considerably among subjects. These preliminary findings suggest that imipramine's mode of antipanic action may be on systems outside of the adenosinergic influence of caffeine.

h. Caffeine-induced Escape from Dexamethasone Suppression. The dexamethasone suppression test (DST) has been suggested as a sensitive and specific tool for the diagnosis of major depressive disorder, melancholic subtype. Because psychiatric patients have been reported to consume excessive amounts of caffeine, because caffeine produces dose-related increases in plasma cortisol, and because the effects of caffeine on the DST had not been previously reported in the literature, the single-dose effects of caffeine 480 mg on the standard dexamethasone suppression test is being investigated in normal volunteers, depressed, and panic disorder patients. Using a single-blind design, an oral dose of caffeine 480 mg or placebo is administered randomly on two separate days at 2:00-2:30 pm the day following the 11:00 pm administration of dexamethasone 1 mg. Test days are separated by at least 48 hours. Blood samples are obtained at 4:00 pm.

Caffeine significantly increases the post-dexamethasone cortisol values. A plasma cortisol level of $> 5 \mu\text{g/dl}$ has been used most commonly to signify nonsuppression. Of the subjects studied to date, 13% have been found to be nonsuppressors on placebo and 31% are nonsuppressors on caffeine. Caffeine-induced nonsuppression has been observed in both depressed patients and normal volunteers. This ongoing study is the first investigation to our knowledge demonstrating that escape from dexamethasone suppression can be induced by caffeine. Of interest, the 480 mg single dose of caffeine given to subjects in this study is roughly comparable to four to five cups of coffee and within the range typically consumed on a daily basis by 20%-40% of the population. Since several lines of evidence suggest that psychiatric patients, particularly depressed and schizophrenic patients, may consume excessive amounts of caffeine, our findings may explain in part the wide variability and discrepant findings in the literature on the DST in psychiatric patients.

9. Panic Disorder and Neuroendocrine Function

a. Corticotropin Releasing (CRH) Test. A CRH test was performed on eight panic disorder patients and compared with 27 normal controls previously studied by the Biological Psychiatry Branch. Compared with normal controls, panic disorder patients had decreased ACTH responses ($p < .01$) and reduced cortisol responses ($p < .05$) to CRH. These findings, published in the Am. J. Psychiatry, suggest that panic disorder patients may have an element of transient hypercortisolemia. The possibility that patients with panic disorder might more readily release CRH in response to environmental perturbation is intriguing in light of the animal data documenting that intracerebroventricular administration of CRH produces a variety of behavioral and physiological changes classically associated with the stress response. Finally, the ability of CRH to increase both locus coeruleus activity and plasma norepinephrine levels is provocative in light of theories implicating increased central noradrenergic activity in the etiopathology of panic disorder.

b. GH-response to Clonidine. Studies using clonidine to assess noradrenergic function continue to be investigated by the Unit on Anxiety and Affective Disorders. Several lines of evidence suggest that the GH-response to clonidine may provide an indirect index of postsynaptic α_2 -adrenergic function. Since noradrenergic dysfunction, particularly noradrenergic overactivity, repre-

sents one of the major current theories of anxiety, the GH-response to clonidine was studied in nondepressed panic disorder patients compared with depressed patients and normal controls triple-matched for age, sex, and menstrual cycle status.

The mean peak growth hormone response to clonidine was significantly decreased in the panic disorder patients and depressed patients compared with normal controls.

Our findings, published in *Biological Psychiatry*, suggest that panic disorder and depressed patients demonstrate a similar blunted growth hormone response to clonidine compared with age- and sex-matched normal controls. These findings are consistent with an emerging body of data suggesting a partial, but incomplete, overlap in the phenomenology, epidemiology, and neurobiology of panic and major depressive disorders.

c. Cortisol Response to Clonidine. Using the cortisol response to clonidine, an α_2 -adrenergic receptor agonist, this study examined the relationship between the noradrenergic system and the HPA axis in patients with major depression, patients with panic disorder, and normal controls.

There was a trend for the three diagnostic groups to differ in baseline cortisol values prior to infusion, with the depressed patients tending to have higher baseline levels.

There was a trend for the three groups to differ in the fall in plasma cortisol in response to clonidine ($F = 2.56$, $p < .10$), with the trend seen between the depressed and panic groups ($p < .06$). However, when the fall in plasma cortisol was expressed as a percentage change from baseline, there was no significant difference among the three groups. In fact, the pre-clonidine plasma cortisol level did not correlate significantly ($p > .10$) with the percentage of change in plasma cortisol level in any of the three groups.

To our knowledge, this was the first study to compare the cortisol response to clonidine across subjects with panic disorder, major depression, and normal controls. While the enhanced cortisol drop following clonidine might suggest a difference in noradrenergic modulation of the HPA axis in depression compared with panic disorder, we believe that the inhibitory effects of clonidine on cortisol secretion may be a less than satisfactory probe of this relationship.

d. Dexamethasone Suppression Test. A standard dexamethasone suppression test was administered to 16 panic patients and 22 normal controls. Using a standard cortisol value of greater than 5 $\mu\text{g/dl}$ to indicate nonsuppression, there was no significant difference between the proportion of panic patients (25%) and normals (14%) with an abnormal test. Moreover, an internal standard for the dexamethasone suppression test was determined using our normal control data. Using a 95% confidence interval (mean + 2 SD) as the criterion for abnormal response (7.1 $\mu\text{g/dl}$), only 6% and 4% of the panic patients and normal controls, respectively, demonstrated cortisol escape from dexamethasone suppression. Our results, pub-

lished in Biological Psychiatry, indicate that panic patients do not respond abnormally to dexamethasone testing when a control group is used to determine the range of normality for a given assay and testing condition.

10. Treatment

a. Panic Disorder

1) Verapamil. Calcium channel blocking agents are widely used in the treatment of cardiovascular disorders. Recent evidence also suggests that these drugs might have positive therapeutic effects in patients with major affective disorders. In a double-blind, placebo-controlled study, we investigated the effects of verapamil, a calcium channel blocker, in the treatment of panic disorder. The study was designed as a double-blind, crossover study and each patient received both verapamil and placebo.

Eleven patients completed the study. A one-way repeated measure analysis of variance (ANOVA) (first placebo versus active drug versus second placebo periods) revealed a significant drug effect on anxiety as measured by the Zung anxiety scale. Posthoc analysis revealed a significant difference between Zung ratings during the placebo pretreatment period and the ratings in the post-treatment period ($p < 0.05$) with a similar trend between the "pretreatment" and the "on-treatment" values ($p < 0.06$). However, there were no significant changes on the Spielberger state anxiety, the NIMH agoraphobia, and the Beck depression scales. Nine of 11 patients (82%) had a decrease in the number of panic attacks during the last four weeks of verapamil treatment compared with the four weeks on placebo preceding verapamil.

These findings, published in the American Journal of Psychiatry, suggest that verapamil has modest anxiolytic and antipanic effects. Additional studies are required to substantiate this finding and compare the efficacy of verapamil to that of drugs such as imipramine, phenelzine, and alprazolam which have a well-established role in the treatment of panic disorder.

2) Nifedipine. Sublingual single dose nifedipine (10-30 mg) was given on three separate occasions to assess the antianxiety effects of this agent in a mixed group of 13 phobic patients with pathological degrees of generalized anxiety. Unlike chronic verapamil treatment, single dose nifedipine failed to demonstrate anxiety reducing effects. Furthermore, in a subset of four patients, the drug failed to show an anxiolytic effect in exposure-related anxiety.

3) Carbamazepine. Our findings of a high frequency of psychosensory symptoms led our unit to investigate the efficacy of carbamazepine in panic disorder patients. While ten of 14 completers demonstrated some improvement on carbamazepine, the overall clinical response was judged to be minimal as reflected by only a small decrement (-4.5 , $p < .02$) on the Zung Anxiety Scale and a nonsignificant change (-1.6 , $p = NS$) on the Spielberger State Anxiety Scale. Forty percent of the patients had a decrease in frequency of panic attacks on carbamazepine, while 50% had an increase and 10% showed no change. Neither the presence of EEG abnormalities nor prominent psychosensory symptoms predicted response to carbamazepine.

This is the first study, to our knowledge, to systematically examine the efficacy of carbamazepine in the treatment of panic disorder using a controlled, double-blind design. Our findings suggest that carbamazepine is of limited value in the treatment of most patients suffering from panic disorder with or without agoraphobia. On most outcome measures, carbamazepine failed to show any benefit over the preceding time period on placebo. While a small decrement was noted on the Zung Anxiety Scale, this was of minimal clinical significance.

4) Clonidine. In previous reports we presented the rationale for investigating the potential antianxiety effects following both an acute intravenous challenge (2 µg/kg) and chronic treatment with clonidine. The following summarizes our experience to date with this agent.

a) Acute Effects. Twelve panic disorder patients and ten normal controls completed a double-blind, controlled clonidine challenge. Placebo failed to produce significant changes in state anxiety in the patients or normal controls (p = NS). While intravenous clonidine also failed to produce changes in state anxiety in the healthy subjects (p = NS), clonidine did produce significant decreases in Spielberger anxiety.

The placebo-corrected changes in anxiety with clonidine (i.e., change in anxiety with clonidine minus change in anxiety with placebo) were also significantly greater in the patients than in the healthy subjects.

b) Chronic Effects. Eighteen patients agreed to participate in the chronic study. Five men and 13 women, with a mean age of (\pm SD) 34 \pm 8.6 years (range 23-58 years), participated in this trial. Clonidine failed to exhibit anxiolytic effects on any of the rating scales. All comparisons were made between pretreatment scores (while on placebo) and scores during the last week of treatment at the highest daily dosage administered. No statistically significant changes were noted on the Zung Anxiety Scale, Global Rating of Anxiety, or the HSCL-90 global severity index, anxiety subscale, phobic subscale, or panic subscale. A trend toward a decrement in anxiety was noted on the Spielberger State Anxiety, but the magnitude of this change was of minimal clinical significance.

b. Social Phobia. To our knowledge, our unit is the first to systematically evaluate the relative efficacy of psychological (i.e., cognitive-behavior therapy [CBT]) versus pharmacological (i.e., placebo vs. phenelzine vs. alprazolam) treatments of social phobia. In a twelve week treatment study, all treatment conditions, including weekly nonspecific encouragement (e.g., placebo), were found to be effective. Preliminary evidence, however, suggests a differential profile of action across the various treatment conditions. First, alprazolam had a more rapid onset of action, although all treatments had demonstrated a comparable level of efficacy by 12 weeks. At a three-month follow-up evaluation after treatment discontinuation, the alprazolam group tended to show a greater percentage of relapse compared with the phenelzine and CBT treatment groups. Of major interest, the physicians who were blinded to the drug conditions found both alprazolam and phenelzine to be significantly superior to placebo on the work and social disability scale.

In a subsequent preliminary study, our Unit has found imipramine to be relatively ineffective in the treatment of social phobia. This same group also has been noted in our preliminary research to have a normal GH response to clonidine, whereas panic disorder patients and patients with major depressive disorder tend to demonstrate a blunted growth hormone response to clonidine. Since a blunted GH response to clonidine has also been demonstrated in recovered depressed patients, these findings, considered together, suggest that a blunted GH response to clonidine may identify a heterogeneous group of tricyclic responders across a wide range of neuropsychiatric conditions.

D. Major Findings (Animal Research)

During the past four years, the Unit on Anxiety and Affective Disorders has established a viable colony of "normal" and "nervous" pure-bred pointer dogs. These dogs offer the advantage of investigating both "normal" behavior and "spontaneously-occurring" (rather than laboratory-conditioned) fear behaviors. The "nervous" line may be particularly useful in the study of several behaviors and characteristics relevant to human psychopathology, including genetically-transmitted inheritance with phenotypic expression of "nervous" behaviors at eight to 12 months of age. This delayed manifestation of pathology in dogs parallels in a similar, temporal fashion, the emergence of agoraphobia in humans during adolescence and early adulthood.

While the colony was being established the development of observation chambers with one-way mirrors and rating scales were developed. Careful and precise techniques for the surgical removal of the whole brain under general anesthesia were also developed and can be reliably performed under suitable conditions. A brain mold for the purebred pointer dog has been developed which allows the Unit to prepare regional brain tissue immediately after surgical removal for later measurement of transmitter levels and ligand binding to various receptors such as α_2 -adrenergic, benzodiazepine, imipramine, opiate, and other binding studies.

The following sections report our preliminary findings with this model.

1. Heritability of Fear Behaviors

During the past year, the Unit has systematically evaluated the validity of the inheritance of nervous behaviors in the A- and E-lines of the Arkansas pure-bred pointer dogs. After breeding dogs from each line at our own facilities, the Unit blindly assessed the behaviors at nine months of age or older. We evaluated "nervous" behaviors using previously validated scales of fear or fear-related behaviors: weighted activity (WA), weighted nervous score (WNS), and new morbidity score (NMS).

The Unit developed several additional scores of fear based on 21 behaviors (i.e., tremor, circling, salivate, etc.) observed under four conditions (dog alone, dog exposed to human sitting on chair, human calling dog, human approaching dog).

On all measures, the offspring of E ("nervous")-line parents had significantly higher nervous scores compared with the offspring of A ("normal")-line parents. There were no sex differences in relation to fear or fear-related behaviors. These data confirm and extend previous observations demonstrating the heritability of fear behaviors in "nervous" pure-bred pointers.

2. Hearing and Non-hearing Pointer Dogs

During the past year, our unit was impressed by a behavioral pattern which was not mentioned in earlier work; namely, that nervous dogs seemed to be less responsive to the presence of a human if the human was not within their field of vision, suggesting a possibility of a hearing deficit. Such a deficit could potentially contribute to or largely determine the aforementioned abnormal behavior in the nervous dogs. Since a hearing deficit has not been previously described in these dogs, we decided to evaluate the hearing status of both nervous and normal dogs in our colony and further assess the relationship between a possible hearing deficit and the abnormal behavior.

The standard brainstem auditory-evoked response (BAER), which has been recently applied to dogs, was used for the assessment of hearing status. Of 16 normal dogs tested, all but one showed normal responses in both ears. One dog (a 4-year-old male) showed a normal response in one ear but no response in the other ear. Direct otoscopic examination gave no clue to the cause of this unilateral deficit. In contrast, the testing in the "nervous" dogs revealed that 20 of 27 dogs had no brainstem evoked response that could be detected in either ear. These dogs were thus considered to be deaf, while the remaining seven dogs had normal responses. However, behavioral ratings revealed that hearing and deaf dogs did not differ in their pathological response to the characteristic fear-provoking stimuli (e.g., human interaction), whereas both hearing and deaf nervous dogs markedly differed from normal dogs on this measure. Thus, regardless of the hearing status, there was a robust difference between nervous and control dogs. That is, these results support a conclusion that hearing status does not effect the behavioral outcome in these dogs and that these traits are not causatively related, although genetic linkage between the behavioral abnormality and the deafness might be expected. These findings will be published in the Journal of Physiology and Behavior. These findings are also of interest in relation to preliminary findings in human panic disorder patients, where we have found a high prevalence of hearing loss in the high frequency ranges.

3. Response to Diazepam and RO 15-1788

The Unit has investigated the effects of diazepam, RO 15-1788, and placebo in ten "nervous" and seven "normal" pointer dogs. Although RO 15-1788 reversed diazepam-induced hind leg ataxia in both lines, there were no significant drug group effects by ANOVA on the previously validated WA, WNS, NMS scales or the ten NIMH subscales of fear behaviors. These data suggest that most abnormal behaviors in this animal model of "anxiety" are unlikely to be mediated by an endogenously-produced, central type, benzodiazepine anxiogenic ligand. Also, the limited effects of diazepam somewhat parallel the relatively poor effects of this agent in the treatment of panic and agoraphobic syndromes in humans.

4. Yohimbine Binding

We studied α_2 -adrenergic receptor binding as determined by [^3H]-yohimbine in platelets and brains of the nervous and normal dogs in our colony. Our findings indicate that α_2 -adrenergic receptor density and affinity are similar in platelets and frontal cortex, but we did not observe significant differences in binding between the two groups of dogs.

5. Adenosine Binding

Since benzodiazepines and adenosine derivatives have marked effects on behavioral arousal, these systems were studied in the brains of both nervous and normal dogs. Adenosine receptors were found to be increased in the hippocampus, and adenosine reuptake sites were found to be increased in the cerebellum of the nervous dogs. No changes were observed in benzodiazepine binding. These findings suggest that adenosine neuromodulatory function might be impaired in the nervous pointer dogs. The report of increased sensitivity to caffeine in panic disorder patients compared with normal controls, and the findings of increased adenosine binding in the hippocampus in the nervous pointer dogs, provide indirect evidence for altered adenosinergic modulation in stress and anxiety syndromes. These findings were published in Clinical Neuropharmacology.

6. Motor Activity

The marked motoric components of the fear response in the nervous dogs (reduced exploratory activity and catatonic freezing) prompted us to investigate spontaneous motor activity in these dogs to determine whether differences in motor activity between the "nervous" and "normal" lines can be demonstrated under more nonstressful conditions over a 24-hour period of time. Our unit studied spontaneous motor activity, using nontelemetric activity monitors, in both nervous and normal pointer dogs.

Data from this study, published in Biological Psychiatry, failed to find any significant difference in motor activity between the nervous and normal pointer dogs. Of interest, however, was the observation that the rest-activity cycle was not evident in either dog line.

II. Proposed Course

Efforts will be expanded to document the phenomenology, natural course, family dynamics, and personality structure of patients with panic disorder. In collaboration with B. Scupi, a Panic Disorder Questionnaire, assessing current and past life experiences, has been developed. This Panic Disorder Questionnaire plus a number of standardized scales including the Parental Bonding Instrument, Retrospective Childhood and Current Fear Scale, Locke-Wallace Marital Satisfaction Inventory, Family Assessment Device, and the Childhood and Adult History Questionnaire will be administered. This study will assess whether retrospective perspectives of early childhood experience and family structure are associated with specific types of symptomatology or predict treatment outcome measures. In collaboration with Dr. J. Maser and B. Scupi, an inventory to assess phenomenology and

natural course of panic and the prevalence of co-morbidity of panic disorder with other psychiatric illnesses has been designed and will be administered to a large sample (> 2000) of subjects worldwide.

Human research conducted by the Unit on Anxiety and Affective Disorders has demonstrated an alteration in neuroendocrine and noradrenergic function in panic disorder patients compared with age- and sex-matched controls. These abnormalities include blunted GH, TSH, and ACTH to clonidine, TRH, and CRH, respectively. Disturbances in noradrenergic function are also suggested by increased DHE binding to platelets and increased sensitivity to the α_2 -adrenergic antagonist, yohimbine. Other lines of evidence reviewed in previous reports also suggest a similarity in neuroendocrine and noradrenergic dysfunction in panic and major depressive disorders. As a result of these findings, the Unit will continue to investigate neuroendocrine and noradrenergic systems across a spectrum of mood and anxiety disorders.

We also intend to expand our research with caffeine. Further delineation of the clinical response to caffeine is indicated because caffeine consumption is correlated with symptoms of generalized anxiety in patients with panic attacks, but not in normal volunteers. Caffeine derivatives also activate noradrenergic activity in animals when iontophoretically applied to the locus coeruleus. Furthermore, caffeine has been shown to antagonize the biochemical and pharmacological effects of benzodiazepines, and alprazolam, a triazolobenzodiazepine, blocks the typical time course of caffeine-induced arousal, panic attacks, and generalized anxiety in humans. Other lines of evidence suggest a major role for adenosine-regulated systems in the mediation of caffeine's psychostimulant properties. All three of these systems have been independently implicated in the neurobiology of anxiety and stress. Caffeine was found by our Unit to significantly elevate measures of the stress-related hormone, cortisol, and induce cortisol escape from dexamethasone suppression. Moreover, panic disorder patients appear to habituate more slowly to repeated caffeine challenges than normal controls. Thus, we intend to extend and expand our ongoing research with caffeine by investigating the behavioral, physiological, neuroendocrine, and biochemical effects of both acute and chronic methylxanthine administration in both animals and humans.

Drug trials with carbamazepine, clonidine, nifedipine, and verapamil have been conducted during the past three years. While preliminary data suggest that each of these agents has modest antianxiety effects in a subgroup of panic disorder patients, none appears to demonstrate the same potency as standard anti-panic agents such as imipramine and alprazolam. A new study investigating the effects of dipyridamole, compared with placebo and imipramine, will be initiated under the direction of Dr. M. Stein. The study of dipyridamole, an agent which blocks the reuptake of adenosine, reflects the Unit's interest in the role of adenosine systems in the neurobiology of anxiety disorders.

In addition to these drug trials in panic disorder, the Unit will expand its clinical studies to include patients with social phobias and obsessive-compulsive disorder. During the past year, the Unit has completed a study investigating the relative efficacy of alprazolam, phenelzine, and cognitive-behavioral treatment of social phobia. This study was conducted in collaboration with C. Shea, a doctoral

candidate in clinical psychology at Catholic University, and Dr. M. Tancer. Preliminary data from this study indicates that alprazolam, phenelzine, and cognitive behavior therapies are all effective in the treatment of social phobia, although in a second study, imipramine appeared to be relatively ineffective. Thus, the identification of both clinical and biological markers of positive drug response in social phobic patients and across a wider spectrum of anxiety disorders will be a major focus of the Unit over the next several years.

In the pointer dog model of "anxiety", the Unit will focus on the behavioral pharmacology of adenosine and adenosine derivatives and xanthine compounds. These experiments in animals will parallel our studies in humans investigating the neurobiology of fear-related behaviors and the mechanisms of antianxiety drugs.

Drug trials with novel antianxiety agents in humans and in "nervous" pointer dogs, in conjunction with concomitant measurements of their neurotransmitter effects, should enhance our understanding of alterations in neuroendocrine and neurotransmitter pathways associated with pathological human anxiety and animal fear, and lead to the development of more potent and specific pharmacotherapies.

III. Significance to Biomedical Research and the Program of the Institute

Several epidemiological surveys have suggested that pathological degrees of anxiety may adversely influence a large segment of our population. Panic disorder, an anxiety syndrome associated with agoraphobia, results each year in the impairment of individuals previously well-functioning and productive. Pathological anxiety has been recently found to be one of the most prevalent mental health problems in this country. The role of anxiety and stress in coronary heart disease and other medical illnesses has been suggested by a number of studies. Moreover, emerging epidemiological and familial data suggest that a subgroup of patients with major depressive illness plus panic attacks may represent an important and distinct subtype of major affective illness. We intend to investigate biological correlates in the plasma and cerebrospinal fluid of this subtype, who may be a greater risk for alcoholism and suicide, compared with patients with major depressive illness without panic attacks. An improved understanding of the clinical and biological aspects of both normal and pathological anxiety is thus critically needed. It is hoped that the developing battery of clinical and biological tests in patients with anxiety and related mood disorders will ultimately provide a clinical and biological profile of these illnesses and lead to more refined subcategorizations, as well as to more selective and efficacious treatment approaches.

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Stein MB, Uhde TW. Autoimmune thyroiditis and panic disorder, Am J Psychiatry, in press.

Klein E, Lenox RH, Uhde TW. alpha-2 Adrenergic receptor binding in platelets and brains of nervous and normal pointer dogs, Psychiatry Res, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00452-13 BP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuroendocrine Studies of Major Psychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Philip W. Gold, M.D., Chief, Clinical Neuroendocrinology Branch, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Clinical Neuroendocrinology

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

20

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been transferred to the newly designated Clinical Neuroendocrinology Branch and is reported under Project #Z01 MH 02432-01 CNE.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01090-12 BP
PERIOD COVERED October 1, 1987 through September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Central Nervous System Functional Anatomy		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Miles Herkenham, Research Psychologist, CNE, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Section on Clinical Neuroendocrinology		
SECTION Biological Psychiatry Branch		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 10	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project has been transferred to the new designated Clinical Neuroendocrinology Branch and is reported under Project #Z01 MH 02433-01 CNE.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00180-06 BP
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychobiology and Treatment of Menstrually-Related Mood Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) David R. Rubinow, M.D., Chief, Unit on Peptide Studies, BPB, NIMH		
Dr. P. Schmidt, BPB, NIMH; M.C. Hoban, BPB, NIMH; Dr. K. Denicoff, BPB, NIMH; Dr. D. Raben, ERRB, NICHD; Dr. R. Elin CPD, CC, NIH; Dr. H. Weingartner, BPB, NIMH; Dr. F. Putnam, St. Elizabeth's Hosp.; Dr. D. Raben, ERRB, NICHD; Dr. N. Hall, George Washington Univ.; Dr. M. Jensvold, BPB, NIMH; Dr. M. Tancer, BPB, NIMH; Dr. M. Stein, BPB, NIMH; Dr. M. Kaliner, LCI, NIAID; Dr. M. Frank, LCI, NIAID; Dr. P. Gindoff, George Washington Univ.		
COOPERATING UNITS (if any) BPB, NIMH; ERRB, NICHD; CPD, CC, NIH; St. Elizabeth's Hospital; George Washington University; LCI, NIAID		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Peptide Studies		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">2</div>	PROFESSIONAL: <div style="text-align: center;">1</div>	OTHER: <div style="text-align: center;">1</div>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The occurrence of dramatic changes in <u>mood</u>, <u>behavior</u>, <u>cognition</u> and somatic functioning in some women in relation to the menstrual cycle has recently been the focus of a great deal of public scrutiny. This project is designed to study the psychobiology and treatment response of women with well-defined <u>menstrually-related mood disorders</u>. The longitudinal screening methods developed by this group are capable of distinguishing women with menstrually-related mood syndromes from those who only believe that they have such a syndrome. With these methods, we have identified the following: 1) the occurrence of the characteristic premenstrual syndrome state during an antiprogesterin-induced follicular phase; 2) a reduction of PMS symptoms during clonidine infusion; 3) menstrual cycle phase-dependent changes in perception of cognitive performance in patients with menstrually-related mood disorders but not controls; 4) a lack of relationship between self-ratings of fatigue and sleep characteristics, but a positive correlation between self-ratings of mood and fatigue; 5) evidence of abnormal basal thyroid measures in 10% of patients with PMS. The goals of this project are to detect and accurately describe menstrually-related mood disorders, explore their pathophysiology and response to pharmacological and environmental manipulation, and to document the relationship between reproductive <u>endocrine</u> change and disorders of mood as a way of further investigating the neurobiology of psychiatric illness.</p>		

I. Project Description

A. Objectives

This project has as its main intent the selection of subjects with carefully documented menstrually-related mood changes who can then undergo psychological and biological evaluation as well as participate in double-blind, placebo-controlled trials of several widely prescribed treatment modalities.

B. Methods Employed

1. Subjects

a. Subjects are self- and physician-referred women between the ages of 18 and 55 who meet study criteria as described in detail in Project #Z01 MH 00180-03 BP.

b. Normal controls for this study include women with no complaints nor evidence of menstrually-related mood disorder and who are without primary psychiatric illness, and women who have complaints of, but no visual analogue scale evidence of, menstrually-related mood changes.

2. Procedures

Phase 1. An extensive screening phase that has been described in detail in Project Z01 MH 00180-02 BP.

Phase 2. This is an intensive psychobiological evaluation phase for patients meeting entry criteria for the study.

a. Patients are given a thorough physical and laboratory examination in order to rule out the presence of unknown medical illness.

b. Ongoing studies of longitudinally obtained basal and stimulated hormonal levels have been previously described in Project #Z01 MH 00180-03 BP. In addition, we are performing the following studies:

1) Psychometrics: Previously described cognitive and mood self-rating batteries are currently being supplemented by psychomotor measures (Purdue Pegboard), determination of menstrual cycle phase-dependent associations, and longitudinal self-evaluation of mood state satisfaction (Drs. H. Weingartner and F. Putnam).

2) Corticotropin releasing hormone (CRH) stimulation tests: With Dr. D. Raben we have performed CRH stimulation tests during the follicular and luteal phase in 12 patients and controls to determine if there is a biological marker for the reported altered stress sensitivity in women with premenstrual syndrome.

3) Clonidine stimulation tests: Because of the hypothesized role of endogenous opiate withdrawal in the precipitation of menstrually-related mood symptoms and a preliminary report of the efficacy of clonidine in the treat-

ment of premenstrual syndrome, we have performed clonidine infusions during the symptomatic and asymptomatic states in order to assess menstrual state-related symptomatic and endocrine response.

4) Immune system: We have investigated T-cell function in relation to menstrual phase in 12 women with premenstrual syndrome and 12 normal controls, in collaboration with Drs. N. Hall and P. Schmidt.

5) Antiprogestin challenge: We have administered an antiprogestin (RU-486) with HCG or placebo in order to blind women to menstrual cycle phase and permit determination of the dependency of mood changes on luteal phase reproductive endocrine change.

6) Menstrual symptom modulation: We are investigating the effects of menstrual cycle phase on symptom intensity or appearance in patients with asthma, hereditary angioedema, obsessive-compulsive disorder, and panic anxiety syndrome (collaborators: Drs. M. Jensvold, M. Tancer, M. Stein, M. Kaliner, and M. Frank). Additionally, studies of postpartum depression (Dr. D. Raben) and menopause and climacteric-related mood disorders (Drs. P. Schmidt and P. Gindoff) have been initiated.

Phase 3. This is a multi-modality treatment phase for patients who have completed Phase 2. Double-blind, placebo-controlled crossover evaluations of progesterone, medroxyprogesterone acetate, pyridoxine, carbamazepine, alprazolam, and nalmefene are currently being conducted. Rationales for the selection of these particular compounds have been previously described. Additionally, a protocol has been submitted for the use of thyroid hormone in the treatment of premenstrual syndrome, based on our observations of frequent thyroid axis abnormalities in patients with premenstrual syndrome as well as the successful elimination of a menstrually-related syndrome in patients with overt hypothyroidism when treated with thyroid hormone. The results with the RU-486 protocol suggest an ability to define subgroups of patients with PMS that may be responsive to customary psychotropic agents. It is believed that these protocols will provide critical information regarding the role of the reproductive endocrine profile during the luteal phase in the production of mood changes that occur during the latter phase of the menstrual cycle.

C. Findings

Results with RU-486 to date show that some women may experience their typical premenstrual syndrome during an experimentally induced follicular phase. Thus, premenstrual syndrome state changes in some women are linked to, but not caused by, luteal phase endocrine changes. The observation that some women have their premenstrual syndrome state successfully eliminated following an RU-486-induced menses suggests at least two types of premenstrual syndromes: those with an autonomous mood disorder linked to the menstrual cycle and those with a menstrual cycle-dependent mood disorder. PMS symptoms have been observed to be significantly diminished within 30 minutes of administration of clonidine (2 µg/kg, i.v). Perceptions of significant cognitive impairment and disturbed sleep have not been corroborated by actual performance measures nor by self-

rated sleep characteristics. These data, along with data from our previously described endocrine studies, are consistent with the hypothesis that premenstrual syndrome represents a biologically facilitated state change, with the altered perceptual characteristics a product of the altered state rather than the normal reproductive endocrine changes. Effects to characterize the biology of premenstrual syndrome have included the performance of TRH and CRH infusions, as well as measurement of progesterone metabolites and immune factors. We have observed abnormalities of basal thyroid hormone indices in 10% of 62 patients and have additionally confirmed and extended our earlier findings of abnormal TSH response to TRH in approximately 35% of patients with PMS. The CRH study has been completed, but the final data are not available for analysis at this time. The same is true for the study of immune factors across the menstrual cycle in patients with PMS and controls and for the measurement of progesterone metabolites. Treatment studies show no consistent benefits of progesterone, medroxyprogesterone acetate, or alprazolam in the treatment of premenstrual syndrome. Finally, early studies examining the role of the menstrual cycle as a modulator of other disorders have revealed no effect of menstrual cycle phase on the incidence or severity of panic attacks in patients with panic anxiety syndrome (collaborator: Dr. M. Stein).

D. Proposed Course of Project

With a group of well-defined patients, we hope to explore the natural course of menstrually-related mood disorders as well as their phenomenology and biological correlates in relation to treatment response. Early endocrine findings will be pursued and specific hypotheses regarding the etiology of premenstrual syndrome (e.g., endorphin addiction/withdrawal, carbohydrate intolerance, electrolyte dysregulation) will be tested with endocrine challenge studies. GnRH and LH pulsatility studies will be initiated to evaluate dynamic function of the reproductive endocrine axis given our prior evidence of absence of abnormalities of basal activity in patients with PMS. Treatment protocols will be completed, and evaluation of putative therapeutic agents will be undertaken. Evaluation of the efficacy of thyroid supplementation in patients displaying an abnormal response to TRH stimulation appears particularly promising. Cognitive testing will be continued with the addition of distractors during testing. We will explore the state- and stress-related characteristics of premenstrual syndrome through implementation of state-dependent learning paradigms and continued performance of CRH stimulation testings. Protocols for the administration of RU-486 and "square wave" progesterone withdrawal will help define, better than any currently available evidence, the nature of the relationship between luteal phase endocrine events and mood changes. These protocols have great explanatory potential because they effectively blind the patient to her position in the menstrual cycle, a methodological problem that was previously uncorrectable. We will continue to expand our investigation of the effects of menstrual phase on mood to include patients hospitalized at the Clinical Center with major affective disorder, panic anxiety disorder, anorexia-bulimia, obsessive-compulsive disorder, as well as patients with hereditary angioedema and asthma. Our early experience with a number of women with these disorders suggests that symptoms may be exacerbated or may cluster during the premenstruum; these clinical impressions require prospective confirmation. Finally, protocols to study women with post-partum and menopausal/climacteric depression have been submitted and approved.

E. Significance to Biomedical Research and the Program of the Institute

Despite the current lack of clear understanding of the nature of the relationship between mood disorders and the menstrual cycle, numerous studies of this phenomenon suggest its importance to the psychiatrist on many levels: practically (as a problem about which the psychiatrist may be called to consult or as a factor that may influence the course of the treatment of patients); heuristically (as a model for learning about state changes, a process of clear relevance to studies of other mood state disorders such as manic-depressive illness or panic anxiety disorder); and conceptually (as a potential means for providing biological-phenomenological isomorphs and further understanding the role of entrainment in episodic or cyclic psychiatric disorders). Menstrually-related mood disorders in their own right are important to better understand, if only for the fact that there are large numbers of women who feel that they suffer from such syndromes and seek treatments that are unproved and potentially dangerous. In addition, it would appear that menstrual cycle phase is a variable that has been all too frequently ignored in studies of traditional psychiatric and medical illnesses. It is our belief, therefore, that this project will provide information that will be of immediate clinical relevance and that will further our understanding of the complex relationship between endocrine system activity and mood.

Publications

Rubinow DR. Practical and ethical aspects of pharmacotherapeutic evaluation. In: Ginsberg BE, Frank-Carter B, eds. PMS: ethical and legal implications in a biomedical perspective. New York: Plenum Press, 1987:47-55.

Rubinow DR, Hoban MC, Grover GN. Premenstrual syndromes - medical and psychiatric perspectives. In: Keyes WR, ed. The premenstrual syndromes. Philadelphia: Grune & Stratton, 1988;27-43.

Both-Ortman B, Rubinow DR, Hoban MC, Malley J, Grover GN: Menstrual cycle phase-related changes in appetite in patients with premenstrual syndrome and in controls. Am J Psychiatry 1988;145:628-630.

Rubinow DR, Hoban MC, Grover GN, Galloway DS, Roy-Byrne PP, Anderson R, Merriam GR: Changes in plasma hormones across the menstrual cycle in patients with menstrually related mood disorder and in control subjects. Am J Obstet Gynecol 1988;158:5-11.

Frankel BL, Rubinow DR. The premenstrual syndromes. In: Howells JG, ed. Modern perspectives in psychiatry. New York: Brunner/Mazel, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00181-05 BP
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Hormonal Studies of Affective Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) David R. Rubinow, M.D., Chief, Unit on Peptide Studies, BPB, NIMH Dr. P. Sunderland, LCS, NIMH; Dr. S. Braca, M, SMRC; Dr. K. Denicoff, BPB, NIMH; Dr. P. Hauser, BPB, NIMH; Dr. P. Lewitt, Lafayette Clinic, Detroit MI; Dr. W. Berrettini, CNG, NIMH; Dr. J. Kleinman, IRP, NIMH; Dr. T. Mellman, BPB, NIMH; Dr. M. Demitrack, BPB, NIMH		
COOPERATING UNITS (if any) BPB, LCS, CNG, IRP, NIMH; SMRC; Lafayette Clinic, Detroit, MI		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Peptide Studies		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Studies of somatostatin and cortisol in relation to <u>affective</u> and other <u>neuropsychiatric</u> disorders have continued. Additionally, other studies have been undertaken including the investigation of <u>progesterone</u> in women with a variety of affective syndromes, beta endorphin in humans and rats administered <u>interleukin-2</u> (IL-2), <u>dehydroepiandrosterone-sulfate</u> in <u>Alzheimer's</u> and <u>AIDS</u> patients, and pituitary cell culture response to IL-2. A) <u>Somatostatin</u> - In human post-mortem regional brain studies, no difference in concentration of somatostatin was observed in the hippocampus, amygdala, or prefrontal region among schizophrenics, suicides, and controls. Post-mortem CSF studies showed significantly increased ventricular CSF somatostatin in Parkinson's and Huntington's patients compared with controls or Alzheimer's patients. B) <u>Cortisol</u> - Salivary cortisol has been used to study patients and controls undergoing caffeine infusion, patients on carbamazepine, and patients with affective disorder, on a longitudinal basis. C) Other assay measures - Assays for plasma beta-endorphin, salivary progesterone, and CSF DHEAS have been developed to study IL-2 and menses-related affective changes, as well as AIDS and Alzheimer's disease.		

I. Project Description

A. Objectives

The goal of this project is to study neuroendocrine and immune soluble products in patients with neuropsychiatric disorders in order to expand our understanding of the mechanisms and significance of reported abnormalities in somatostatin, cortisol, and immune system activity in affective illness.

B. Methods Employed

1. Subjects

a. Subjects include inpatients on NIMH clinical units meeting criteria for major depressive disorder, Alzheimer's disease, Huntington's dementia, Parkinson's disease, and multiple sclerosis.

b. Normal controls are volunteers selected from the normal volunteer program at the NIH. Neurological controls were employed for studies of patients with multiple sclerosis.

2. Procedures

Lumbar punctures are performed to obtain CSF samples for somatostatin, CRF, and other related CNS peptides/neurotransmitters. Regional analysis of brain somatostatin is performed in post-mortem human brain samples. Brain slices in animals are punched and assayed for somatostatin content. Whole brains are dissected and radioactivity measured in animal lymphokine studies. Animals are cannulated to measure blood hormone levels following IL-2 administration. Rat pituitary glands are cultured to measure stimulating effects of IL-2.

II. Findings

A. Somatostatin

An attempt is currently being made to replicate Dr. Hauser's preliminary findings of a decrease in CSF somatostatin in patients with multiple sclerosis relative to neurologic controls, as well as an increase in CSF somatostatin in patients with multiple sclerosis during clinical remission. No differences were observed in somatostatin concentrations in several brain regions (hippocampus, amygdala, or prefrontal region) in human post-mortem specimens in schizophrenics, suicide victims, or accident victims (collaborators: Drs. J. Kleinman and S. Braca). Somatostatin concentrations from post-mortem ventricular samples were higher in patients with Parkinson's disease (5) and Huntington's disease (5) compared with Alzheimer's disease (12) or controls (14) (collaborator: Dr. W. Berrettini). No difference was observed between controls and Alzheimer's patients, raising questions about the utility of post-mortem ventricular CSF values as an indicator of brain somatostatin content. Further study of lumbar CSF somatostatin in Alzheimer's patients (collaborator: Dr. T. Sunderland) revealed a significant reduction compared with age-matched controls.

B. Cortisol

Significant increases in cortisol were observed in anticipation of a caffeine infusion in patients with anxiety disorder but not controls (collaborator: Dr. T. Mellman). Carbamazepine was found to induce escape from dexametha-

sone suppression as measured by salivary cortisol levels in nine patients tested on and off medication. Longitudinal evening salivary cortisol measurement showed no correlation between mood ratings and salivary cortisol values. Escape from dexamethasone suppression was observed with salivary cortisol measures in an AIDS patient who developed an interferon-induced depressive syndrome (collaborators: Drs. M. Demitrack and K. Denicoff). Plasma cortisol concentrations were found to significantly increase after IL-2 administration and to show profound enhancement of stimulation following repeat challenge with IL-2.

C. Progesterone

We have demonstrated the practical utility of the salivary progesterone measure in the evaluation of reproductive endocrine function across the menstrual cycle. This measure is currently being used to document ovulation in patients with menstrually-related and climacteric-related mood disorders.

D. beta-Endorphin

An assay for plasma beta-endorphin has been developed and tested and has shown highly significant increases following administration of IL-2. This assay is being adapted for use in rats to permit investigation of the acute vs. chronic effects of IL-2 in beta-endorphin secretion in vivo as well as in pituitary cell culture.

III. Proposed Course of Project

We hope to: 1) employ salivary cortisol measures to characterize mood state switches that occur in patients with affective illness in ambulatory settings as well as patients undergoing immunotherapy; 2) investigate in a larger study the relationship between cognitive performance and CSF somatostatin in patients with Parkinson's dementia (collaboration with Dr. P. LeWitt); 3) develop assay techniques to permit evaluation of the contribution of somatostatin fragments to the total syndromal alterations in CSF somatostatin; 4) identify the mechanism of the sensitization observed in the endocrine response to IL-2 by developing in vivo rat and in vitro pituitary cell culture models.

IV. Significance to Biomedical Research and the Program of the Institute

Depression-related dysregulation of somatostatin and cortisol may provide a window into the central neurochemical lesions responsible for depression. Further, specific behavioral or physiological disturbances (e.g., cognitive impairment or cortisol dysregulation) may be products of abnormal neuroendocrine activity. It may prove to be the case that depression-related reductions in somatostatin are mechanistically relevant to depression-related disturbances in hypothalamic-pituitary-adrenal activity, the most commonly reported biological abnormality in depression. Determination of the mechanisms of the profound behavioral and cognition altering effects of interleukin-2 would fill a major gap in our knowledge of the ways in which the immune system can regulate central nervous system activity. Further study may not only enhance our knowledge of the neurobiology of depression, but may, as well, more generally inform us about the relationship between hormones and human behavior.

Publications

Rubinow DR: Somatostatin in depressive disorders. Actualites Psychiatriques, 1987;4:67-74.

Kahn JP, Rubinow DR, Davis CL, Kling M, Post RM: Salivary cortisol: A practical method for evaluation of adrenal function. Biol Psychiatry 1988;23:335-349.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00182-05 BP
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Behavioral Medicine		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) David R. Rubinow, M.D., Chief, Unit on Peptide Studies, BPB, NIMH Dr. K. Denicoff, BPB, NIMH; Dr. M. Jensvold, BPB, NIMH; Dr. S. Rosenberg SB, NCI; Dr. P. Brouwers, Georgetown Univ.; Dr. C. Lane, LIR, NIAID; Dr. S. Pillemer, ARB, NIADDD; Dr. N. Hall, Georgetown Univ., Dr. P. Schmidt, BPB, NIMH; Dr. D. Lango, FCRF, NCI; Dr. G. Peck, DB, NCI; Dr. E. Sternberg, NB, NIMH; Dr. R. Cohen, LCM, NIMH; Dr. C. Ollo, BPB, NIMH; Dr. M. Heyes, LNP, NIMH; Dr. M. Tancer, BPB, NIMH; Dr. M. Kaliner, LCI, NIAID; Dr. M. Demitrack, BPB, NIMH		
COOPERATING UNITS (if any) BPB, LCM, LNP, NB, NIMH; SB, FCRF, D, NCI; LIR, LCI, NIAID; A&R, NIADDD; Georgetown University, Washington DC.		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Peptide Studies		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Ten protocols are currently active and conducted out of the Consultation-Liaison Service-based <u>behavioral medicine</u> research program. These protocols examine the phenomenology and biological correlates of illness or treatment-induced mood, behavioral, and cognitive changes. The protocols address such areas as: a) the effects of previous psychiatric history on the psychiatric morbidity associated with certain diseases and their treatment; b) the psychiatric phenomenology of certain diseases and their treatment; c) the treatment response characteristics of psychiatric disorders associated with diseases or their treatment; d) biochemical factors that may serve as predictive diagnostic markers for illness or for treatment-associated mood/behavioral or cognitive syndromes; e) the effects of mood state alterations on <u>immunologic function</u> . Significant findings to date include demonstration of the following: 1) confirmation of earlier described significant neuropsychological cognitive impairment in patients with AIDS compared with sero-positive patients or controls; 2) evidence of stimulation of ACTH, beta-endorphin, and cortisol by IL-2 administration, with profound sensitization of endocrine stimulation following an absence of IL-2; 3) preliminary confirmation of alpha-delta intrusion in patients with fibromyalgia; 4) absence of evidence of increased prevalence of suicidal acts or ideation in patients with Darier's disease.		

I. Project Description

A. Objectives

This project has as its main intent the identification of mood and cognitive symptoms that appear in the context of specific medical illnesses and their treatment, determination of the relationship between these symptoms and both the primary medical disorder and prior psychiatric history, and utilization of the occurrence of these symptoms in a medical context as models for the occurrence of similar symptoms in a primarily psychiatric context.

Protocols

Active:

- 1) Neuropsychiatric dysfunction in patients with Acquired Immune Deficiency Syndrome (AIDS).
- 2) Assessment of PET abnormalities of patients with Acquired Immune Deficiency Syndrome (AIDS) (collaborators: R. Cohen, C. Lane).
- 3) Assessment of neuropsychiatric concomitants of metoclopramide administration.
- 4) The effect of hypnotically-induced affect on immune function in normal subjects.
- 5) Investigation of suicidality in patients with Darier's disease (collaborators: G. Peck, K. Denicoff).
- 6) The endocrine effects of interleukin-2/lymphokine-activated killer cell therapy (collaborators: S. Rosenberg, K. Denicoff).
- 7) Mood and cognitive toxicity of gamma-Interferon administration in seropositive patients (collaborators: C. Lane, M. Demitrack, K. Denicoff).
- 8) Fibromyalgia (collaborators: S. Pillemer, K. Denicoff).
- 9) Assessment of psychiatric morbidity and suicidality in patients with scleroderma (collaborators: E. Sternberg, M. Jensvold).
- 10) The prevalence of anxiety disorders and menstrually-related symptom exacerbation in patients with asthma (collaborators: M. Tancer, M. Jensvold, M. Kaliner).

Completed:

- 1) The effect of thyroid replacement and withdrawal on cognition and mood in patients with carcinoma of the thyroid.
- 2) Neuropsychiatric effects of alternate day steroid administration in patients with systemic lupus erythematosus.

Planned:

- 1) Conditioned immunosuppression and immunoenhancement in cancer patients.
- 2) Development of endocrine correlates of the "intensive care unit syndrome".
- 3) The effects of psychostimulants on depression in the medically ill.
- 4) Eye tracking abnormalities in seropositive and AIDS patients compared with seronegative high risk patients and controls.
- 5) The effects of cytoxan on IL-2 stimulated ACTH and beta-endorphin.

B. Methods Employed

1. Subjects

a. Subjects are NIH patients who are referred for participation in these protocols by collaborators from the Institute responsible for the primary care and treatment of these patients.

b. Controls for the individual studies are selected in a way that allows for stratification of populations with respect to the relevant variables under study.

2. Procedures

a. Psychiatric Diagnostic Evaluation

The primary methodology employed is that of evaluating the psychiatric history of all subjects and their families utilizing a semistructured psychiatric interview, the Schedule for Affective Disorders and Schizophrenia (SADS-L), which provides information from which an RDC diagnosis can be made.

b. Longitudinal Evaluation

Most studies utilize a "self as own control" design employing longitudinal assessment of mood ratings, physical symptoms, and cognitive performance. Detailed description of methodologies employed can be found in Project #Z01 MH 00182-02 BP.

c. Laboratory Assessment

Urine and/or blood samples are collected in order to permit evaluation of those biological substances believed to be related to the development of affective or cognitive disturbances.

3. Findings

1) We have demonstrated stimulation of ACTH, beta-endorphin, and cortisol in the plasma following i.v. administration of IL-2. Additionally, we have observed a profound enhancement of this endocrine stimulation following the absence of IL-2 treatment from one week to three months prior to the second course of treatment. This enhancement was not seen following up to 12 repeated sequential doses of IL-2. This is the first demonstration of endocrine sensitization of this order of magnitude and provides compelling evidence for the interaction of the immune and neuroendocrine systems. Preliminary analysis also suggests that the extent of the ACTH stimulation observed during the second course of treatment may be associated with the course of illness following the second IL-2 treatment course (collaborators: Drs. K. Denicoff and S. Rosenberg).

2) Attempts to recruit patients for the fibromyalgia study have revealed the exceedingly low prevalence of primary fibromyalgia; i.e., only one of ten patients who appear to meet screening criteria for fibromyalgia actually have primary (as opposed to secondary) fibromyalgia. Alpha-delta intrusion has been observed electroencephalographically in the four patients tested who do meet criteria for fibromyalgia. Additionally, trials of the antidepressant amitriptyline are being undertaken in the treatment of the symptoms of fibromyalgia as well as in the reversal of the observed EEG abnormalities (collaborators: Drs. S. Pillemer and K. Denicoff).

3) We have observed significantly elevated levels of the tryptophan metabolite and excitatory neurotoxin, quinolinic acid, in the spinal fluid of patients with AIDS compared with normal controls. These findings will be pur-

sued in a larger group of patients with AIDS as well as seropositive/AIDS negative patients and seronegative controls (collaborators: Drs. C. Lane and M. Heyes).

D. Proposed Course of Project

The studies noted above will be continued until adequate numbers of subjects are obtained. Attempts to reverse the IL-2-stimulated increase in ACTH, beta-endorphin, and cortisol with cytoxan will be undertaken in a pilot study in patients receiving IL-2/LAK therapy. Animal and in vitro studies pursuing this finding are described in Project #Z01 MH 00181-04 BP. Studies investigating the phenomenologic, somnographic, and treatment response characteristics of patients with fibromyalgia will be continued. Studies of seronegative, seropositive, and AIDS patients have been expanded to include PET scanning and eye tracking in addition to neuropsychological measures. In such a fashion we hope to be able to identify early markers of central nervous system dysfunction associated with HIV infection. Completion of these descriptive studies should permit design of focused investigations of the neurobiology of specific mood, behavioral, and cognitive disorders. Finally, studies are currently being undertaken to investigate the efficacy of stimulants in the treatment of depression in the medically ill, describe the development and endocrine concomitants of the "intensive care unit syndrome", and investigate the possible existence of conditioned immunosuppression and immunoenhancement in cancer patients.

E. Significance to Biomedical Research and the Program of the Institute

The studies in this project are hypothesis-generating as well as hypothesis-testing. Thus, they should not only help to expand the behavioral phenomenology of many medical disorders, but should, as well, suggest optimal studies for the application of modern neuroscientific techniques to disorders of regulation of mood and cognition. Detection of conditioned immunosuppression in patients should have profound effects on both our understanding and treatment of many medical disorders. Identification of markers for the acquisition and progression of HIV-related central nervous system dysfunction should greatly advance our understanding of the etiology and consequences of AIDS-related and possibly HIV-related dementias. The utilization of medical disorders as models for the development of mood and cognitive disturbances in the context of biological dysregulation should clarify the meaning of those biological alterations already observed in psychiatric disorders.

Publications

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00147-13 BP
PERIOD COVERED October 1, 1987 through September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Behavioral and Physiological Effects of Brain Peptides and Other Psychoactive Compounds		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Agu Pert, Ph.D., Chief, Unit on Behavioral Pharmacology, BPB, NIMH Lorna Estal, BPB, NIMH; Andrea Mele, BPB, NIMH; P. Glue, LCS, NIAAA; R.J. Weber, LC, NIADDK; K.C. Rice, LC, NIADDK; B. DeCosta, LC, NIDDKD; D. Nutt, LCS, NIAAA; R. Rothman, IRP, NIMH; S.R.B. Weiss, BPB, NIMH; C.C. Chiueh, LCM, NIMH, N. Ostrowski, LCM, NIMH		
COOPERATING UNITS (if any) BPB, CNB, LCM, NIMH; LC, NIADDK; LC, NIDDKD; LCS, NIAAA;		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Behavioral Pharmacology		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 3.5	PROFESSIONAL: 2.5	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Repeated administration of <u>cocaine</u> to rats produced <u>behavioral sensitization</u> but did not alter <u>dopamine</u> levels or <u>dopamine metabolites</u> in response to cocaine challenge as revealed with <u>microdialysis</u> procedures. <u>In vivo microdialysis</u> revealed differential effects of cocaine on <u>dopamine release</u> following direct applications to the frontal cortex, nucleus accumbens, or striatum. Acute <u>ECS</u> enhanced the release of dopamine in the frontal cortex but not any other region, while chronic ECS appeared to elevate basal dopamine levels in all three dopamine terminal areas. <u>Morphine</u> applied to the striatum or nucleus accumbens increased the release of dopamine, with the latter structure being more sensitive. Morphine applied unilaterally to the substantia nigra (SN) or ventral tegmental area decreased dopamine release in the ipsilateral striatum and nucleus accumbens, respectively. The <u>kappa opiate</u> agonist <u>U-50,488</u> increased nociceptive thresholds in a stereoselective manner following systemic injections. The inactive and active enantiomers were equally effective in modifying nociceptive thresholds following i.v. injections, indicating that the analgesic effects of this class of opiates are mediated through the spinal cord or through peripheral mechanisms. No tolerance developed to the depressant effects of U-50,488 on <u>locomotor activity</u>, while the excitatory effects increased in intensity with repeated injections. The mesolimbic dopamine system does not mediate the excitatory effects of kappa opiates. <u>Diuresis</u> induced by kappa agonists is mediated through the CNS. <u>PCP-induced</u> activation of the SN resulted in both increases and decreases in the metabolic activity of diencephalic and mesencephalic structures. Alterations in the metabolic activity of structures caudal to the SN are related to the motor asymmetries produced by intranigral PCP. PCP and other noncompetitive <u>NMDA antagonists</u> altered metabolic activity in a variety of structures. Injections of <u>morphine</u> into the periaqueductal gray matter as well as <u>electrical stimulation</u> of this structure produced a <u>suppression</u> of NK cell activity. <u>Opiate receptors</u> in the SN are located on <u>striato-nigral terminals</u>.</p>		

I. Project Description

A. Objectives

1. Analysis of the Effects of Psychoactive Drugs on Dopamine Function Using in vivo Microdialysis

Repeated administration of the sympathomimetics cocaine and amphetamine results in behavioral sensitization that is characterized by an augmentation of locomotor activity and stereotypy with repetitive injections. The neurochemical mechanisms underlying this phenomenon are not entirely understood. Since these sympathomimetics act by increasing the release and preventing the reuptake of dopamine (DA), it is possible that sensitization is related to an increase in the efficacy of these mechanisms. We have evaluated the effects of chronic cocaine exposure on cocaine-induced increases in dopamine and dopamine metabolites in the nucleus accumbens and striatum of rats using microdialysis procedures. In these studies, rats were pretreated daily for one week with either 30 mg/kg cocaine or a similar volume of saline. One day following termination of treatment, the animals were anesthetized and placed into a stereotaxic frame. Two mm microdialysis probes were introduced into either the striatum, nucleus accumbens, or frontal cortex. Artificial CSF was pumped through the probes and dialysate collected every 15 min. Following baseline stabilization, various concentrations of cocaine were added to the dialysate. The collected dialysate was assayed for dopamine and dopamine metabolites using HPLC with electrochemical detection immediately following collection. In a separate study, two groups of rats were treated chronically with saline for seven days, as previously described. A third group received cocaine. On day eight, one of the groups treated with chronic saline, and the group treated with chronic cocaine, were injected with cocaine 30 minutes prior to sacrifice. The other chronic saline group received saline. The brains were removed and the striatum dissected. The tissue homogenates were centrifuged, and dopamine and dopamine metabolite levels were analyzed by HPLC-EC.

Cocaine is thought to induce its psychomotor stimulant as well as its reinforcing actions through the mesolimbic and mesocortical dopamine systems and its stereotypic actions through the nigrostriatal systems. Cocaine has been most commonly reported to act at presynaptic receptors, blocking reuptake of dopamine, although at higher doses it may also increase dopamine release. Most studies have used indirect methods of estimating these effects, either in tissue slices or synaptosomal preparations, or by looking at tissue levels of dopamine and its metabolites. Furthermore, there is little information regarding regional differences in cocaine-induced alterations in dopamine function. We have compared the effects of focally applied cocaine on three brain regions that are innervated by dopamine terminals using in vivo microdialysis procedures. Microdialysis probes were introduced into either the frontal cortex, striatum, or nucleus accumbens of anesthetized rats. Various concentrations of cocaine (0.01-10 mM) were introduced through the probes, and alterations in dopamine and dopamine metabolites measured in the dialysate with HPLC methodology.

There is considerable controversy regarding the effects of opiates on dopamine function. Early studies suggested that opiates block dopaminergic function,

while more recent studies have indicated that opiates may actually enhance dopamine release. We have initiated a series of studies with microdialysis procedures to determine the precise loci of action of opiates in modulating dopamine activity. Anesthetized rats were implanted with unilateral microdialysis probes in the striatum or the nucleus accumbens. Following baseline stabilization, various concentrations (0.1-10 mM) of (+) or (-) morphine bitartrate were introduced into the dialysate. The subsequent dialysate was analyzed for dopamine and dopamine metabolites with HPLC-EC. In another series of studies rats were implanted with cannulae guides aimed for the SN or the ventral tegmental area (A-10). Microdialysis probes were then introduced into the ipsilateral striatum or nucleus accumbens, respectively. Following baseline stabilization, the animals were injected in the ventral tegmentum (VTA) or SN with various doses of (+) and (-) morphine bitartrate (.5-25 nmoles). Dialysate from the nucleus accumbens or striatum was assayed for dopamine and dopamine metabolites following either VTA or SN injections of morphine.

2. Effects of Electroconvulsive Shock on Dopamine and Dopamine Metabolites as Assessed with Microdialysis

There is a considerable literature on the effects of acute and chronic electroconvulsive shock (ECS) on brain neurotransmitters and neurotransmitter metabolites. All of these studies, however, have been done in tissue homogenates, the results of which are often difficult to interpret. We have utilized in vivo microdialysis to monitor alterations in dopamine and dopamine metabolites following acute and chronic ECS in three regions of the rat brain. In the first series of studies, microdialysis probes were introduced into either the frontal cortex, nucleus accumbens, or striatum of anesthetized rats. Following stabilization of the dopamine levels, rats were shocked for 0.5 seconds with 80 mA of AC current delivered across the ears. Dopamine and dopamine metabolite levels were monitored for one hour following ECS. The same animals were allowed to recover from the anesthetic and then given daily ECS sessions for one week. At the end of this period, the animals were anesthetized again and placed in the stereotaxic frame. Microdialysis probes were introduced into the same structures as before, but in the opposite side. Dopamine and dopamine metabolites were monitored before and after ECS, as before.

3. Psychopharmacology of kappa Opiate Receptor Agonists

a. Locomotor behaviors A great deal of effort has been devoted to the development of nonaddictive opiate analgesics. Originally, it was hoped that certain benzomorphan analogs that interact selectively with kappa opiate receptors might have the desired characteristics to suit this need. Over the past year, we have systematically characterized the pharmacological effects of this unique series of opiate agonists in the rat following intracerebral injections of the highly selective kappa agonist U50,488. In our previous studies, we found that the active enantiomer (S,S) or U50,488 was much more active than the inactive enantiomer (R,R) in several behavioral tests following central administration. One of the most profound effects of this agonist was on locomotor output. We have further characterized the effects of U50,488 on locomotor activity and attempted to define the neurohumoral circuits involved in these effects.

Acute injections of opiate agonists selective for the mu opiate receptor produce an initial depression of locomotor activity which is followed by excitation. In order to determine whether the selective kappa opiate receptor agonist also shared similar properties, rats were injected systemically with various doses of (S,S) or (R,R) U50,488 and placed in locomotor activity monitors for 75 minutes. Following chronic administration of mu opiate agonists, animals became tolerant to the locomotor depressant effects while the excitatory actions increased with repetitive injections. In order to determine whether chronically administered kappa agonists produce similar effects, rats were injected systemically with the (S,S) enantiomer for ten days. Opiate receptor involvement of the effects of (S,S) U50,488 were ascertained by co-administration of naloxone or the specific kappa receptor antagonist MR 2266. It is suggested that the locomotor stimulatory effects of morphine are due to activation of the mesolimbic dopamine pathways. In order to determine if the excitatory effects of the kappa agonist U50,488 were also mediated through the mesolimbic dopamine pathways, rats were lesioned in the nucleus accumbens with 6-OHDA and compared with sham operated controls following injections of the (S,S) enantiomer.

b. Analgesia. Although kappa receptor-specific opiate agonists produce antinociceptive effects in a variety of tests, there is still confusion regarding the precise neural focus of their actions or pharmacological specificity. In these studies, we have evaluated and compared the antinociceptive properties of the active and inactive enantiomers of U50,488 following either systemic or i.v. injections.

In the initial studies, rats were injected with various doses of (S,S) U50,488 i.p. (5-50 mg/kg) and then tested in the hot-plate, tail-flick, or paw pressure tests at certain time intervals following injection. In order to ascertain whether the antinociceptive effects were mediated by the CNS, rats were implanted with cannulae guides aimed for the lateral ventricle. Following recovery from surgery, the animals were injected with various doses of (S,S) or (R,R) U50,488 in the ventricles and tested in the three antinociceptive tests, as before.

c. Diuresis. One of the most selective effects of kappa opiate agonists is on water balance and retention, probably through their actions on ADH release. It is not known, however, whether these effects are mediated directly through the CNS or indirectly through peripheral mechanisms. In our initial series of studies, rats were implanted with chronic indwelling cannulae guides in the lateral ventricle. Following recovery, the animals were injected intraventricularly with various doses of (S,S) or (R,R) U50,488 (25-100 nmoles) and then placed in metabolic cages for five hours. Urine output was measured two and five hours following injection.

3. Effects of Phencyclidine and Other Noncompetitive NMDA Antagonists on Brain Function

Phencyclidine (PCP) is a powerful psychotomimetic substance that produces psychopathological effects that mimic the primary symptoms of schizophrenia. Many of the effects of PCP have been thought to involve dopaminergic mechanisms.

In our previous studies, we identified the SN as an important focus for the behavioral actions of PCP. In order to further define the functional outputs of the nigrostriatal system that are activated by PCP, rats were implanted with unilateral cannulae guides aimed for the SN. Following recovery, the animals were injected in this structure with 25 nmoles of PCP, and i.v. with 100 uCi/kg of [14 C]-2-deoxyglucose (2-DG). The brains were prepared for autoradiographic analyses using standard procedures. Rotational behavior was also assessed with an automated rotation monitor.

In addition to PCP, MK-801 as well as SKF 10,047 also appear to have similar psychotomimetic properties. We have compared the effects of these three non-competitive NMDA antagonists on the metabolic activity of rat brain. Rats were injected systemically with either PCP, (+) SKF 10,047, (-) SKF 10,047, MK-801, or saline. Thirty minutes later, all animals were injected with 2-DG. Standard procedures of visualizing 2-DG uptake were employed. The effects of the drugs on locomotor activity were measured in automated activity monitors for 45 minutes following the injections of 2-DG.

4. Modulation of the Immune Function by the CNS

We have previously localized the immunosuppressant of morphine to the periaqueductal gray matter (PAG) of the mesencephalon. Injections of morphine into this structure produced a significant suppression of NK cell activity and T-cell proliferation in response to mitogens, while injections into a variety of other brain structures were without effect. We are currently attempting to define the neural circuitry involved in modulating immune functions. Rats were implanted with bipolar electrodes in the PAG of mesencephalon. Following recovery, the animals were stimulated intermittently in the PAG with bipolar pulse trains for ten minutes.

Behavioral reactions to the stimulation were noted and antinociceptive effects quantified. Three hours following the stimulation, the rats were sacrificed, their spleens removed, and natural killer cell activity measured.

5. Functional Outflow of the Nucleus Accumbens

It is certain that the nucleus accumbens represents a critical focus for the excitatory effects of sympathomimetic compounds. We have evaluated the functional outputs of this structure following direct activation with the sympathomimetic amphetamine. Rats were implanted with unilateral or bilateral cannulae guides aimed for the nucleus accumbens. Following recovery, the animals were injected with 15 nmoles of d-amphetamine sulfate and 100 uCi/kg of 2-DG i.v. Immediately following injections of 2-DG, the rats were placed in locomotor activity monitors for 45 minutes. At the end of this period, the animals were sacrificed and their brains removed and frozen. Twenty micron coronal sections were cut through each brain. Every tenth section was mounted on a glass microscope slide. The slides were apposed with x-ray film for ten days. A Spatial Data Systems image analyzer was used to quantify the optical densities in various brain structures.

6. Autoradiographic Distribution of Opiate Receptors in Relation to Dopamine Neurons

The localization of opiate receptors on dopamine neurons has still not been resolved. Opiate receptors are found in high concentrations in the SN as well as the striatum and nucleus accumbens. The opiate receptors in the terminal fields of the dopamine pathways appear to be located on dopamine terminals since 6-OHDA lesions of the medial forebrain bundle or SN reduce binding of opiate ligands in the striatum as well as the nucleus accumbens. The location of opiate receptors in the dopamine cell body regions has not been resolved although it has been assumed that they are found on dopamine cells. Autoradiographic studies combined with lesioning procedures were conducted to examine opiate receptor binding relative to dopamine neurons. Afferent and efferent connections of the SN were eliminated unilaterally in rats with 6-OHDA lesions of the medial forebrain bundle or electrolytic lesions of the internal capsule which carries the striato-nigral afferents. Ten days following the lesions, the animals were sacrificed and the brains prepared for autoradiographic analysis.

B. Major Findings

1. Dialysis Studies

Findings from microdialysis studies revealed no differences in basal levels of dopamine or its metabolites in either the striatum or nucleus accumbens between rats exposed chronically to cocaine and those exposed to saline. Furthermore, while 1 mM (-) cocaine HCl introduced through the dialysis probe produced a dramatic increase of dopamine in the dialysate, no difference in either dopamine or dopamine metabolites appeared between the two groups of rats to the cocaine challenge in either structure. In the tissue level studies, dopamine levels in the striatum were no different in animals pretreated with cocaine for one week from those given a single injection. In addition, ratios of DOPAC/DA, HVA/DA, and 3-MT/DA were similar among all three groups, suggesting that there were no differences in enzyme activity. Overall, it would seem that one week of cocaine pretreatment does not produce any change in presynaptic DA function in either the striatum or nucleus accumbens, assessed by tissue levels of DA and metabolites, metabolite/DA ratios, or cocaine-induced release of dopamine and dopamine metabolites. These findings indicate that cocaine-induced sensitization involves mechanisms that are postsynaptic to dopamine terminals.

Dopamine release was seen in both the striatum and nucleus accumbens at the lowest doses of cocaine used in this in vivo study (0.01 mM) compared with the limited dopamine responses seen in vitro, where concentrations greater than 10 mM may be required to obtain an effect. This is in contrast to the effects of d-amphetamine which has equivalent effects on dopamine responses both in vivo and in vitro. This suggests that factors other than blockade of dopamine reuptake and/or enhancement of dopamine release are important in the mechanism of cocaine. Although there are differences between the mechanisms of action of amphetamine and cocaine (e.g., cocaine-induced responses are Ca^{2+} -dependent while amphetamine-induced responses are not), it would seem that the integrity of other modulatory neuronal systems are more critical for cocaine-induced dopamine release than for amphetamine.

Dopamine responses to all concentrations of cocaine in the striatum were of greater magnitude than those in the nucleus accumbens or frontal cortex. The ratio of DA released in the striatum compared with the nucleus accumbens was not constant, but increased with the concentration of cocaine used. Cocaine infusion into the frontal cortex induced increases in dopamine levels that were not dose-dependent and were also considerably lower in magnitude than those in the striatum or nucleus accumbens. These findings indicate that there are differential dopamine responses to cocaine in these three areas and suggest the involvement of different mechanisms. One possibility is the involvement of different dopamine uptake processes in the three brain regions. It is known, for example, that the nucleus accumbens has only low affinity dopamine uptake sites, while the striatum has both low and high affinity sites.

Direct infusions of (-) morphine bitartrate into the striatum through the dialysis probes enhanced DA release in the striatum while the inactive enantiomer had little effect. The nucleus accumbens proved to be even more sensitive to the direct applications of morphine. Injections of morphine into the SN, however, produced a stereospecific decrease in DA release in the ipsilateral striatum. Similar effects were seen in the nucleus accumbens following injections of morphine into the ventral tegmental area. It appears that the stimulatory effects of opiates on DA release are mediated through opiate receptors located on DA terminals. Actions in the cell body regions appear to exert opposing effects.

Acute ECS was found to enhance the release of dopamine and norepinephrine in the frontal cortex. There was relatively little effect on DA or DA metabolites in the striatum, and dopamine levels fell in the nucleus accumbens. There was little effect on dopamine, norepinephrine or metabolite responses to ECS following chronic exposure in any brain region. Chronic ECS, however, did appear to alter the basal levels in all three regions, especially the nucleus accumbens and frontal cortex.

2. Psychopharmacology of kappa Opiate Agonists

Systemic injections of (S,S) U50,488 produced an initial dose-dependent decrease in locomotor output which was followed by excitation. No tolerance was observed to the locomotor depression following chronic administration, while the excitatory effects increased with repetitive injections. Neither MR 2266 nor naloxone antagonized the excitatory effects of U50,488. 6-OHDA lesions of the nucleus accumbens also failed to alter the locomotor stimulatory effects. The effects of systemically administered U50,488 on locomotor activity appear to be mediated through different mechanisms from those mediating the effects of centrally administered U50,488 or other opiate receptor agonists and do not involve mesolimbic DA.

Systemic injections of (S,S) U50,488 (the active enantiomer) increased reaction latencies in the hot-plate, paw-pressure, and tail-flick tests. The inactive enantiomer (R,R) U50,488 did not alter the reaction times in any test. Surprisingly, however, the effects of the active enantiomer were relatively resistant to antagonism by either naloxone or MR 2266. Intraventricular injections of (S,S) U50,488 (100 nmoles) also increased reaction latencies in the hot-

plate and paw-pressure tests. The inactive enantiomer, however, was equally effective. Nor-BNI (a selective kappa receptor antagonist) did not antagonize the effects of (S,S) U50,488 in any test. These findings suggest that kappa agonist antinociception does not appear to involve supraspinal mechanisms, but it probably mediates either peripherally or at the spinal level. The active enantiomer of U50,488 produced a dramatic dose-dependent increase in urine output while the inactive enantiomer had no effect. Studies are underway to define the mechanisms and neural loci underlying this effect.

3. Noncompetitive NMDA Antagonists and Brain Function

Intranigral PCP was found to produce profound rotational behavior contralateral to the injection. This behavior was accompanied by increases in the uptake of 2-DG in the habenula, medial forebrain bundle, superior olive, caudate/putamen, striatal fundus, pedunculopontine tegmental nucleus, and the cerebellum, while decreased uptake was observed in the cingulate cortex, medial thalamus, periaqueductal gray matter, substantia nigra, retrorubral field, and the superior colliculus. Decreases in SN metabolic activity resulted in both increases and decreases in the metabolic activity of mesencephalic and diencephalic structures. Since we have previously shown with lesioning techniques that only structures caudal to the SN are critically involved in mediating the effects of intranigral PCP, it appears that alterations in metabolic activity of mesencephalic and pontine regions are related to the motor asymmetries induced by this manipulation.

Systemic injections of the various NMDA antagonists had a biphasic effect on locomotor output. Generally, there was an initial depression of locomotor output followed by locomotor excitation. MK-801 was the most potent drug tested in this regard. Concomitant with increases in locomotor behavior, significant increases in metabolic activity were found in the anterior and posterior cingulate cortex, anteroventral, ventromedial and posterior thalamic nuclei, dorsal and ventral hippocampus, and substantia nigra (zona reticulata) following MK-801. PCP also enhanced metabolic activity in the anterior and posterior cingulate and the SN (ZR) as well as the caudate nucleus. Unlike MK-801, however, PCP decreased metabolic activity in the thalamic nuclei. In general, both enantiomers of SKF 10,047 reduced the metabolic activity of a variety of structures, including the hippocampus.

4. CNS Modulation of Immune Function

Injections of morphine into the periaqueductal gray matter (PAG) produce a significant suppression of NK cell activity and T-cell proliferation in response to nitrogen, while injections into a variety of other brain structures were without effect. Stimulation of the ventromedial aspects of the PAG produced profound aversive reactions in the animals, which were also accompanied by a significant suppression of NK cell activity. The PAG may represent an important integrative region for mediating the aversive consequences of exogenous stressors on immune function.

5. Output of the Nucleus Accumbens

Injections of amphetamine into the nucleus accumbens produced significant increases in locomotor activity that were also accompanied by alterations

in the uptake of 2-DG in a variety of structures. Increases in uptake were seen in the ventral pallidum, nucleus of the diagonal band, PAG, retrorubral field, red nucleus, SN (ZR), pontine reticular nuclei, and ventral tegmental area. Decreased uptake was seen in frontoparietal cortex, red nucleus of the stria terminalis, medial thalamus, and lateral habenula. It is interesting to note that the predominant changes were found in the terminal areas of the nucleus accumbens efferent projections. The alterations seen in the mesencephalic and thalamic regions are probably related to the locomotor stimulatory effects of amphetamine.

6. Opiate Receptor Localization

Internal capsule lesions produced significant decreases in opiate receptor binding in the dorsal (pars compacta) and ventral (pars reticulata) SN, the ventral tegmental area, and the medial terminal nucleus of the accessory optic tract. In contrast, elimination of the DA cells produced minimal change in [^3H]naloxone binding in the same regions. These data suggest that the primary site of opiate receptor binding is on nigral afferents, and not on DA neurons.

Significance to Biomedical Research and the Program of the Institute

Chronic use of psychomotor stimulants in man is known to result in a variety of dysfunctional mood and cognitive states. Elucidation of the mechanisms involved should be important to an understanding of the acute and long-term changes in affective behavior associated with cocaine use. Moreover, stimulant-induced behavioral sensitization shows cross-reactivity to a variety of stressors and, as such, should provide a relevant model for the progressive evolution of behavioral pathology in response to the same stimulus over time. This model may thus help elucidate principles and mechanisms included in the progressive evolution of recurrent affective illness.

Since opiate alkaloids produce some of the most profound behavioral and physiological effects, endogenous opiates (which are mimicked by the alkaloids) must serve an important role in regulating emotions as well as physiological and sensory processes. The use of our newly developed autoradiographic procedures, which allow a measurement of ongoing activity in these systems, may reveal the functional significance of opiate pathways in brain.

Phencyclidine is an increasingly abused substance. Furthermore, phencyclidine, as well as other noncompetitive NMDA antagonists, produces effects in man very similar to some of the primary symptoms of schizophrenia. For these reasons, it is valuable to understand the mechanisms of action of this class of compounds.

Besides endorphins, the brain contains numerous other peptides which undoubtedly serve important regulatory functions. It is important to identify the distribution of binding sites for other substances in brain and to analyze their physiological and behavioral effects with micro-injection mapping techniques. Alterations in the activity of these systems may underlie a number of neurological and psychiatric disorders.

Proposed Course of Project

1. Over the past year, we have devoted considerable effort to establishing in vivo microdialysis capabilities in both anesthetized and awake rats. Over the next several years, we plan to utilize this new technology to evaluate the effects of psychoactive drugs on dopaminergic, noradrenergic, and serotonergic functions in both anesthetized and awake animals. Special emphasis will be placed on correlating neurochemical changes in defined brain regions with specific behaviors.
2. Behavioral sensitization has implications for understanding psychiatric disorders as well as phenomena associated with drug abuse. Attempts will be made to evaluate neurochemical and neuropharmacological alterations following chronic sympathomimetic exposure with in vivo microdialysis and receptor autoradiographic techniques.
3. Further research will be directed at defining neural circuits affected by competitive as well as noncompetitive NMDA antagonists using 2-DG methodology.
4. In vivo autoradiographic procedures will be used to define endorphinergic circuitry activated by various behavioral and physiological manipulations.
5. Functional metabolic activity of specific brain circuits will be observed during various behavioral states and following focal injections of various drugs using 2-DG procedures.
6. Studies will be initiated to define specific brain circuits that regulate immune function using microinjection, electrical stimulation, and lesioning procedures.
7. Studies will be conducted to localize opiate receptor subtypes on serotonergic, noradrenergic, and dopaminergic systems.

Publications

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Pert A, Clarke PBS. Nicotinic modulation of dopaminergic neurotransmission: functional implications. In: Martin WR, Van Loon GR, Davis DL, Iwamoto, eds. Tobacco smoking and health: a neurobiological approach. New York: Plenum Press 1987;169-190.

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Clarke PBS, Skirboll L, Pert A. Anatomical and electrophysiological evidence for the nicotinic cholinergic innervation of the dopaminergic neurons of substantia nigra pars compacta. Neuroscience, in press.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00400-06 BP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protein Phosphorylation in Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Jitendra Patel, Visiting Associate, Unit on Neurochemistry, BPB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Neurochemistry

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been discontinued.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01831-12 BP

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic and Clinical Studies of Neuronal and Glial Enolases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Paul J. Marangos, Ph.D. Chief, Unit on Neurochemistry, BPB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Neurochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

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- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been discontinued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01833-08 BP
PERIOD COVERED October 1, 1987 - September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Adenosine Receptors in the CNS</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Paul J. Marangos, Ph.D. Robert M. Post, M.D. Dag Von Lubitz, Ph.D. Chris Gleiter, M.D. Susan R.B. Weiss, Ph.D. Jean-Luc Daval, Ph.D. Takashi Nakajima, M.D. Jurgen Deckert, M.D. Philip F. Morgan, Ph.D.	Chief, Unit on Neurochemistry, Chief Neurologist Staff Fellow Staff Fellow Guest Researcher Fogarty Visiting Fellow Guest Researcher Fogarty Visiting Fellow	BPB, NIMH BPB NIMH Georgetown Univ. LCS, NIAAA BPB, NIMH BPB, NIMH BPB, NIMH BPB, NIMH BPB, NIMH LCS, NIAAA
COOPERATING UNITS (if any) BPB, NIMH; LCS, NIAAA; Georgetown University		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Neurochemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.7	PROFESSIONAL: 0.6	OTHER: 1.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) During the past year, increasing attention has been focused on the pre-clinical evaluation of various <u>adenosinergic</u> systems including both the <u>adenosine receptor</u> and the <u>adenosine uptake site</u> . In particular, we have focused on <u>seizure mechanisms</u> as regards the interaction between <u>carbamazepine</u> and the adenosine receptor, as well as the relationship between <u>caffeine</u> and carbamazepine. We have also shown that adenosine agonists can afford marked neuroprotective effects in <u>cerebral ischemia</u> . Studies have also been initiated regarding the <u>proto-oncogene product c-fos</u> and its relationship to seizures. We have shown that <u>electroconvulsive seizures</u> can induce c-fos <u>mRNA</u> using <u>northern blots</u> of brain mRNA. Studies are in progress attempting to relate c-fos expression to the <u>kindling</u> process. We have initiated studies of the <u>N-type calcium channel</u> using [¹²⁵ I]- <u>conotoxin</u> as a ligand probe. Studies are in progress evaluating the response of N-type channels to various seizure paradigms. We are exploring the potential of developing new <u>anticonvulsant drugs</u> targeted at the N-type calcium channel.		

I. Project Description

A. Objectives

The Unit on Neurochemistry of the Biological Psychiatry Branch has, for the past eight years, focused on characterizing the adenosinergic neuromodulatory system in brain. During this period, we have studied the biochemical, anatomical and pharmacological properties of both the adenosine receptor and the adenosine uptake site. In so doing, we have shown that, by radioreceptor binding techniques, multiple receptor and uptake site populations exist in brain. We have also generated systems where uptake and receptor sites can be clearly discriminated using specific ligands for each (see Z01 MH 01833-07 BP).

The adenosine system is highly relevant from the clinical standpoint in that adenosine has been implicated as being a natural sedative agent that tends to maintain neurons in a homeostatic condition. Adenosine agonists inhibit virtually all calcium-dependent neurotransmitter release, thereby establishing this simple purine as a major modulator of brain function. Our efforts have mainly been focused on the development of new adenosinergic drugs relevant to both psychiatry and neurology for which we are convinced there is great potential. It is highly likely that new sedative anxiolytic and anticonvulsant agents can be developed based upon their activity on specific subsets of either the receptor or the uptake site.

During the past year, we attempted to investigate several rather intriguing aspects of adenosinergic mechanisms in the CNS which include: a) the elucidation of the carbamazepine interaction with adenosine receptors; b) the characterization of neuronal N-type calcium channels, which may be modulated by adenosine receptors; and c) the relationship of the proto-oncogene product c-fos to various seizure phenomena and the adenosine receptor. Specific questions involve whether some of the anticonvulsant properties of carbamazepine can be a result of adenosine receptor interactions. Also, are seizures modulated by neuronal N-type calcium channels, and does the c-fos proto-oncogene product couple membrane events to nuclear events in neurons?

B. Methods Employed

Radioreceptor assays, scintillation counting, northern blotting, gel electrophoresis, animal seizure paradigms (ECS, drug-induced kindling), quantitative densitometry, receptor autoradiography, transient cerebral ischemia in gerbils.

C. Results

Several major quantitative autoradiography studies have been completed during the past year by Drs. Daval and Deckert. In the first, we studied the effect of global cerebral ischemia on hippocampal adenosine receptors. It has been shown that CHA (an adenosine agonist) has marked protective effects in cerebral ischemia, indicating that the adenosine agonists may prove to be important in the treatment of stroke patients. We have shown, autoradiographically, that CHA preserves the CA-1 pyramidal cells in ischemic (20 minutes) gerbil hippocampus. Without CHA treatment, the adenosine receptors in both the stratum oriens and the stratum radiatum decrease drastically three days post-ischemia compared with the CHA-treated animals. These results are to be submitted to Journal of Neuroscience.

The second autoradiography study dealt with the comparison of adenosine receptor upregulation in chronic caffeine and chronic carbamazepine-treated rat brain. Here, Drs. Daval and Deckert showed that virtually identical upregulation patterns were observed throughout the brain. We also showed that adenylyl cyclase levels were also similarly increased by both caffeine and carbamazepine. This indicated that the adenosine receptor increases observed represented functional sites. This study has been submitted to Epilepsia.

The studies described in last year's report relating to the characterization of carbamazepine interactions at the adenosine receptor have all been accepted for publication and further studies are now in progress relating to the mechanism of carbamazepine as regards the upregulation of adenosine receptors. In these studies, we will compare single dose carbamazepine with caffeine as they relate to adenosine receptor upregulation. Preliminary studies have already shown that one dose of carbamazepine is sufficient to increase adenosine receptors.

Our efforts in the area of molecular neurobiology during the past year have begun to produce interesting results. Drs. Nakajima, Daval, and Gleiter have performed some interesting studies regarding the proto-oncogene product c-fos and DNA binding protein that is thought to be involved in calcium-dependent alterations of neural physiology. We have shown that a single electroconvulsive seizure (ECS) causes a rapid and transient expression of c-fos mRNA. Of particular interest in this study was the observation that even the earclip control animals (no ECS) manifested a slight c-fos expression. This indicates that either stress or pain can modulate the levels of this nuclear protein. This study has been submitted for publication to the Neuroscience Letters. Further studies are planned involving chronic ECS and the response of c-fos.

We have also recently shown that subconvulsant doses of caffeine can upregulate c-fos expression in mouse brain. Interestingly, this can be partially blocked by the central benzodiazepine receptor antagonist, RO15-1788. Other agents such as benzodiazepine agonists, adenosine agonists, and peripheral benzodiazepine receptor ligands are also being evaluated in this paradigm. These studies represent the first subconvulsant pharmacologic modulation of c-fos expression to date.

C-fos studies planned for the coming year will evaluate c-fos expression during kindling, and we will also attempt to map the localization of c-fos mRNA using in situ hybridization strategies. It will be of particular interest to determine whether kindled animals are sensitized as regards c-fos expression. These experiments hold the promise of providing new and key insights regarding the molecular substrates of kindling.

It is well established that adenosine inhibits the calcium-dependent release of very many neurotransmitters. We have tried, but with limited success for years, to establish a relationship between calcium antagonist binding sites in brain and adenosine receptors. These studies involved the L-type calcium channel, which predominates in muscle. Recently, it has become possible to

study N-type calcium channels using the 27 amino acid toxin called conotoxin. The N-type channel has been shown to be the one involved in neurotransmitter release and therefore may be the more relevant entity as regards the adenosine receptor. Also, this may be the relevant site for modulating seizures. Therefore, the study of N-type calcium channels using conotoxin can be of considerable value in various seizure paradigms. We have successfully incorporated the [125] I conotoxin binding assay into our laboratory and are planning a variety of studies regarding seizures and their effect on this site in brain. Initial studies have involved ECS at the chronic and acute level and will compare both the L- and N-channel. Future studies will involve kindled animals and the adaptive responses of both the L- and N-channel in various brain areas. Autoradiographic studies are also planned where potential regional alterations in N-type channels will be sought.

We believe there is a significant potential for the development of new anti-convulsant strategies based on the modulation of N-type calcium channels. It has been rather conclusively shown that these are the channels that mediate neurotransmitter release and are probably much more relevant to synaptic function than L-type channels. It is also likely that N-type channels are relevant to kindling and learning mechanisms.

D. Significance to Biomedical Research and the Program of the Institute

The studies described should provide basic mechanistic insights regarding sedation, seizures and various post-ischemic processes. The N-type calcium channel may well prove to be a particularly important target site for the development of a new generation of anticonvulsants that prove to be highly selective. Also, the c-fos proto-oncogene product may provide new molecular insights relating to the mechanism of seizures and kindling, both of which are of significant clinical importance.

E. Proposed Course of the Project

These studies should continue for at least one year.

PUBLICATIONS

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Bisserbe JC, Deckert J, Marangos PJ. Autoradiographic distribution of [³H]dipyridamole binding sites in guinea pig brain, Neurosci Lett 1986;66:341-5.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02432-01 CNE

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Stress-Response Neurohormones in Health and Disease: Basic and Clinical Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Philip W. Gold, M.D., Chief, Clinical Neuroendocrinology Branch, NIMH

Others: Dr. M.A. Kling	Senior Staff Fellow	CNE	NIMH
Dr. H.A. Brandt	Senior Staff Fellow SBP	LCS	NIMH
Dr. H.J. Whitfield	Medical Staff Fellow	CNE	NIMH
Dr. M.A. Smith	Pratt Fellow	CNE	NIMH
Dr. K. Kalogeras	Visiting Fellow	CNE	NIMH
Dr. T.D. Geraciotti	Medical Staff Fellow	CNE	NIMH

(continued on page 2)

COOPERATING UNITS (if any)

Developmental Endocrinology Branch, NICHD

LAB/BRANCH

Clinical Neuroendocrinology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

20.0

PROFESSIONAL:

15.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our clinical studies show that melancholic depression is associated with concomitant activation of the principal effectors of the stress response, namely, the CRH and locus ceruleus-norepinephrine (LC-NE) systems, while atypical depression seems associated with pathological inactivation of these neuronal elements. Further evidence of functional linkage between the CRH and LC-NE systems derives from our work showing that NE is a potent stimulus to CRH release and data that the icv administration of CRH markedly and kindling preferentially increases LC glucose utilization. To support a role for CRH in the periodic course of affective illness, we have shown that the limbic convulsant procaine produces dose-dependent activation of pituitary-adrenal function in volunteers and releases CRH from hypothalamic organ culture (an effect inhibited by carbamazepine). Moreover, preliminary data suggest that the icv administration of CRH antisera attenuate electrically-kindled seizures. While anorexia nervosa and major depression share a common defect in CRH regulation which we postulate contributes to many of their common features, patients with the eating disorders show additional abnormalities in mechanisms subserving hunger and satiety which may confer pathophysiological specificity. Hence, bulimics show a marked decrease in the plasma levels of the anorexogenic peptide cholecystokinin in response to food intake in association with decreased satiety, and increased levels of CSF peptide YY, known to significantly increase hunger. In a primate model of depression, we have shown that adverse maternal-offspring interactions significantly inhibit head circumference, limb length, and weight, and predispose to the development of a depressive-like syndrome in subordinate animals and those subjected to brief isolation. As an adjunct to our previous work showing that the CRH stimulation test is helpful in the differential diagnosis between major depression and Cushing's disease, we now have shown that the CSF/plasma ACTH ratio can distinguish between these illnesses in over 95% of cases. In studies employing a chronically implanted lumbar drain, we have shown neuropeptides are secreted into human CSF in a pulsatile fashion with a circadian variation in pulse frequency and amplitude. Because patients with affective illness often show abnormalities in both thyroid and adrenal axes, we explored the relationship between these axes and showed that experimentally-induced hypothyroidism decreased the synthesis and release of CRH while experimentally-induced hyperthyroidism produced the opposite results.

(Continued from page 1)

Z01 MH 02432-01 CNE

Others:	Dr. M.A. Demitrack	NSRA Fellow	CNE	NIMH
	Dr. M. Heatherington	Visiting Fellow	CNE	NIMH
	Dr. A. Spalter	Visiting Fellow	CNE	NIMH
	Dr. A. Calogero	Guest Researcher	DEB	NICHD

Introduction:

The Clinical Neuroendocrinology Branch has attempted to pursue a series of coherently related clinical and pre-clinical studies to explore the endocrine functions of the brain, the mechanisms of physical and emotional stress, and the relevance of stress-responsive neurohormones to the pathophysiology of psychiatric illnesses such as major depression and anorexia nervosa. In clinical studies, we have attempted to focus our studies in atypical and melancholic depression on interactions between the CRH and locus ceruleus-norepinephrine systems utilizing paradigms such as the continuous monitoring of lumbar CSF for thirty hours via an indwelling lumbar drain and the assessment of basal and stimulated norepinephrine spillover into arterial blood. Pre-clinical studies exploring the relationship between the CRH and locus ceruleus-norepinephrine systems also focus on the regulation and expression of the glucocorticoid receptor and its role in mediating counter-regulation to these systems; methodologies include *in situ* hybridization, high resolution autoradiography, immunocytochemistry, positron emission tomography, and behavioral pharmacology. In keeping with our recent emphasis on anorexia nervosa and bulimia, we are further attempting clinical and biochemical definition of these disorders with clarification of their relationship to the affective disorders; as a corollary, we are exploring neural mechanisms subserving hunger and satiety which may confer pathophysiological specificity upon the eating disorders as well as possibly influencing their marked female preponderance. Our interest in Cushing's disease as an illness sharing biochemical and clinical features with major depression has led to elucidation of their differential pathophysiology and the most sensitive diagnostic test employed in their differential diagnosis; further studies on differential pathophysiological mechanisms in these two disorders and efforts to improve the means for establishing their differential diagnosis remain as important goals of our group. An additional effort has been made to develop an animal model of depression in marmosets bred in our laboratory, followed from birth through puberty with documentation of mother-offspring interactions, and subjected to social stressors such as the competition for dominance in large peer groups or social isolation. Additional projects summarized in this report include efforts to explore interactions between the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-adrenal thyroid axes, and studies of the relevance of stress-responsive neuromodulators to post-partum depression. A corollary of the latter project is an emphasis on the study of the endocrine functions of the placenta, particularly on the placental-mediated CRH-induced hypercortisolism of pregnancy, and the possible consequences of suppression of the central CRH system and steroid withdrawal in post-partum states.

The following section is organized into separate consideration of eight interrelated major project areas currently under investigation by our group.

1. Studies of the interactions among stress-responsive systems, particularly the CRH and locus ceruleus-norepinephrine systems, and the relevance of these interactions to melancholic and atypical

depression; as a corollary, study of the role of the glucocorticoids as counter-regulatory CRH and locus ceruleus-norepinephrine system, with emphasis on the regulation and expression of Type 1 and Type 2 glucocorticoid receptors in hippocampus, hypothalamus, and locus ceruleus.

Progress: On the basis of our data obtained over the past several years in patients with major depression, we recently proposed that melancholic depression is characterized by concomitant activation of the major components of the stress response, namely the CRH and LC-NE systems (Gold, *et. al.*, Clinical and Biochemical Manifestations of Depression: Relationship to the Neurobiology of Stress, N. Engl. J. Med., in press); moreover, we wrote that it was this concomitant activation that conferred many of the clinical manifestations of hyperarousal characteristic of this illness. An additional point emphasized in this work was that the activation of these components of the stress response represented a cause rather than a consequence of the depressive process, with a possible underlying biological vulnerability reflected in a deficient capacity of the glucocorticoids to perform their principal role during the stress response (namely modulation of the gene(s) called into play to counterregulate its major components, especially CRH and LC-NE activation). Included among the many supporting lines of evidence advanced by our group was a series of almost three dozen studies exploring the clinical applications and pathophysiologic relevance of CRH, ultimately leading to the conclusion that hypercortisolism in melancholic major depression reflected a defect at or above the hypothalamus resulting in the hypersecretion of CRH; moreover, we have recently demonstrated a strong positive correlation between CRH and several indices of noradrenergic hyperactivity in patients with melancholic depression.

In contrast to the insomnia, anorexia, and intense anxiety which are the cardinal manifestations of melancholic depression, "atypical" depression seems its antithesis, being associated with hyperphagia, hypersomnia, and evidence of pathological inactivation. In this regard, we have shown that patients with Cushing's disease, whose hypercortisolism we have shown derives from a pituitary rather than a central abnormality, most typically show evidence of atypical depression in association with significant decreases in CSF CRH and CSF NE. We presume that the evidence of a decrement in the function of the CRH and LC-NE systems in Cushing's disease in association with symptoms of pathological inactivation reflect excessive glucocorticoid counterregulation of the major components of the stress response. The finding that atypical depression in Cushing's disease, in contrast to melancholic depression, seems associated with a pathological decrement rather than accentuation in CRH and LC-NE function is of great theoretical interest. Hence, though affectively ill patients with atypical depression have been reported to show "normal" pituitary-adrenal function and an absence of evidence of adrenergic activation, pathological decrements in the functional activity of these systems are extremely difficult to detect unless relatively elaborate studies are specifically designed to do so. In this regard, such studies are currently underway in our group. An additional theoretical point raised by clinical observations that

some patients during the course of periodic depression show melancholic depression at one phase and an atypical depression at another is that the apparently antithetical pattern in CRH and LC-NE function in these subgroups may not be entirely unrelated, but coherently related, dysregulations in a given system.

To complement our clinical studies, we undertook a series of studies to explore the regulation of CRH synthesis and release in brain, as well as to explore possible relationships between the CRH and LC-NE systems. One of our first preclinical studies exploring this issue was an *in vitro* study which showed unequivocally that NE was a potent stimulus to the release of CRH from *in vitro* hypothalamic culture (Calogero, Chrousos, and Gold, J. Clin. Invest., in press), a finding complementing Rita Valentino's work that CRH was a potent stimulus to the LC firing rate. Further work in our laboratory, conducted by Dr. Miles Herkenham and colleagues, showed that the icv administration of labeled CRH was taken up in a strongly preferential fashion by LC neurons. Additional work showed that CRH given icv markedly increased LC glucose utilization, with a concomitant decrease in glucose utilization in LC terminal fields such as hippocampus. Utilizing the technique of *in situ* hybridization, a technique now mastered by several members of our group with the generous help of Dr. Scott Young (representing an ideal union between neuroanatomy and molecular biology) we showed that although CRH content in LC goes up during stress, that CRH mRNA is undetectable there, hence clarifying the question of whether CRH and NE were co-contained in LC neurons. Further work showed that one possible source for CRH in the LC was the nucleus paragigantocellularis, one of the few sources in addition to the PVN of afferent input into the LC. Additional studies utilizing *in situ* hybridization showed that acute and chronic stress produced differential changes in CRH and tyrosine hydroxylase mRNA in brain. Hence, acute stress produced a concomitant increase in PVN CRH mRNA and LC TH mRNA while adaptation developed during the application of chronic stressors for two weeks. These data have implications for normal adaptive mechanisms called into play during chronically stressful situations and further support the idea that the sustained activation of components of the stress response in melancholic depression represents a pathologic dysregulation rather than an appropriate adaptive mechanism. Parenthetically, we are currently in the process of exploring the effects of acute and chronic stressors in these parameters in Maudsley rats, a strain bred for hyperresponsiveness to stressful input.

The data advanced in our laboratory is compatible with two recent studies suggesting intimate linkage between CRH and LC-NE systems indicating that the functional activity of one is contingent on participation by the other. In the first of these, it was shown that a weak CRH antagonist, alpha helical CRH, completely abolished the effects of hemorrhage and nitroprusside on LC firing, while propranolol inhibited the effects of icv CRH to increase locomotor activity. We are currently in the process of evaluating the effects of more potent CRH antagonists and of CRH antisera on a variety of physical and emotional stimuli known to have predictable effects of LC firing, as well as

studies aimed at exploring the extent to which CRH in the CSF is a physiologically meaningful stimulus to LC function.

A corollary to our studies exploring interactions between the CRH and LC systems are studies aimed at exploring the possibility that deficient glucocorticoid counterregulation of the stress response leads to its excessive and prolonged activation in melancholic depression. In our N. Engl. J. Med. paper, we speculated that a failure of glucocorticoids to adequately modulate the TH gene on the short arm of chromosome 11 could lead to a situation where, over the course of a lifetime, frequent and prolonged stress responses could occur as a consequence of a lower threshold and/or inadequate counter-regulation, leading to possible sensitization of underlying limbic substrates (see below).

To explore the effects of glucocorticoid and glucocorticoid antagonists on interaction function, we have applied *in situ* hybridization to examine the location and quantity of mRNA for a variety of hormone species (e.g., AVP, OT, CRH, POMC, CCK, NPY, SS, TRH, TH, DYN, ENK) in disparate locations including hippocampus, hypothalamus, and if relevant, in LC. Parenthetically, because so little is known about the distribution of neurons that express and synthesize these substances, some brains have been sectioned for reconstruction and mapping of normal patterns so that we are currently engaged in a massive data-gathering phase that will have many long-term benefits.

An additional strategy to explore the potential role of glucocorticoids on CNS function has been pursued by Dr. Harvey Whitfield in collaboration with Dr. Cary Weinberger of the Laboratory of Cell Biology. Specifically, Dr. Whitfield has been interested in characterizing the physiological significance of the so-called Type 1 or mineralocorticoid receptor which is heavily expressed in several brain regions, particularly hippocampus. This receptor not only binds mineralocorticoid with high affinity but also shows a higher affinity for glucocorticoids than the classic Type 2 glucocorticoid receptor. To study the functional significance of this receptor, Dr. Whitfield has constructed two genes which code for a constitutively active mineralocorticoid receptor and one which binds glucocorticoids but fails to bind normally to the DNA binding region. Dr. Whitfield is now in the process of screening a genomic library of human brain for sequences which would hybridize with 5' and 3' flanking sequences of the mineralocorticoid receptor gene. The purpose of this component of the project is as a preparation for insertion of these mutant genes into transgenic mice for site specific expression in brain. It is hoped that by studying the behavior and physiology of the transgenic mice that a further elucidation of the physiological and pathophysiological relevance of mineralocorticoid receptor gene expression in brain can be elucidated.

2. Study of the relevance of stress-responsive neurohormonal systems to the natural history of periodic affective disorder:

Progress: Our group has become increasingly invested in exploring the potential role of CRH in the periodic course of major depression ever since

we first demonstrated that CRH produces limbic seizures which cross-sensitize with electrically kindled seizures. To further explore the potential role of CRH in kindling and sensitization phenomena in clinical studies, we administered procaine to volunteers and patients with affective disorder to monitor pituitary-adrenal and behavioral responses. We noted that procaine produced dose-dependent increases in ACTH and cortisol secretion in both patients and volunteers in association with mood and/or psychosensory changes. To explore the potential mechanism of procaine's effects upon the pituitary-adrenal axis, we assessed the effects of this agent as well as other local anesthetics such as lidocaine and cocaine on *in vitro* release of CRH from hypothalami and of ACTH from cultured pituicytes. We noted that procaine produced a dose-dependent increase in CRH release but had no effect on the pituitary corticotroph. Interestingly, the effect of procaine was blocked by carbamazepine but not by a number of other agents, including alpha 1 and alpha 2 blockers, serotonin 1B blockage, and nicotinic and muscarinic blockade. To further explore the potential role of CRH on neuronal kindling, we pretreated rats with icv CRH antisera. This intervention was able to profoundly attenuate the development of electrically induced kindling. Taken together, these data suggest that repeated activation of the CRH system over the course of recurrent depression could sensitize underlying limbic substrates to influence the natural history of the illness and represents the strongest data to date implicating a stress-responsive neuropeptide in a process which is known to show cross-sensitization with stressful stimuli.

To follow up on these findings, Drs. Mark Smith and Harvey Whitfield have embarked upon a series of studies to explore the effects of electrical kindling on the expression of genes of interest utilizing *in situ* hybridization. Some of the questions to be addressed in this study include the effects of kindling on CRH mRNA in disparate sites such as PVN, nucleus paragigantocellularis, and nucleus hypoglossus. Probes for a variety of other genes, as noted above, will also be applied. A specific focus of the study will be to explore the evolution in the pattern of gene expression as the process of kindling proceeds to completion.

3. Further clinical and biochemical definition of anorexia nervosa and bulimia, with clarification of their relationship to affective disorders; as a corollary, consideration of possible neural mechanisms subserving hunger and satiety which may confer pathophysiological specificity upon the eating disorders, as well as possibly influencing their marked female preponderance.

A. Clinical Studies:

Progress: In our clinical work, we have recently published a series of papers in the N. Engl. J. Med. and J. Clin. Endocrinol. Metab. implicating CRH and arginine vasopressin in the pathophysiology of hypercortisolism in anorexia nervosa.

The evidence regarding the role of CRH in the hypercortisolism in major depression and anorexia stems from a series of studies exploring the

integrity of glucocorticoid negative feedback upon the pituitary in these disorders, the similarity between CRH induced-hypercortisolism in controls and the pattern and magnitude of hypercortisolism in these disorders, and the levels of CSF CRH and their relationship to pituitary-adrenal function. The defect in CRH hypersecretion is significantly more pronounced in anorexia nervosa and may contribute, in part, to the refractoriness these patients show to psychotropic agents. From a series of studies in laboratory animals and primates on the effects of centrally-administered CRH, the data suggest that the common defect in CRH regulation in our patients could account for a number of their common manifestations.

Our data regarding arginine vasopressin secretion in anorexia nervosa has shown complete disruption of osmoreceptor function in this disorder in association with marked hypersecretion of centrally directed arginine vasopressin. These data are of interest in light of studies showing synergy between arginine vasopressin and CRH at the pituitary corticotroph cell and evidence that arginine vasopressin delays the extinction of learned behaviors acquired during aversive conditioning. In this regard, anorexic patients show a markedly exaggerated sense of the adverse consequences of food consumption.

In contrast to the series of studies indicating that the pathophysiology of hypercortisolism in depression and anorexia nervosa reflect a common defect in the regulation of the CRH system, our data indicate that abnormalities in the secretion of arginine vasopressin differ in the two disorders. Hence, in contrast to evidence of disrupted osmoreceptor function associated with increased centrally directed arginine vasopressin secretion in patients with eating disorders, patients with major depression (non-psychotic) show osmotically mediated but subnormal vasopressin secretion during osmotic stimulation, secondary either to a decreased sensitivity of the osmoreceptor or a delay in the osmotic threshold for vasopressin secretion. This decrease in plasma vasopressin secretion is associated with a corollary decrease in vasopressin secretion into the CSF. An exception to the pattern of decreased vasopressin secretion into the plasma and CSF in depression is the markedly enhanced plasma and CSF vasopressin secretion which we have consistently noted in psychotically depressed patients. These data are of potential interest in light of the fact that psychotic depressives can show the same kind of obsessive perseverative focus on issues relating to loss and personal deficiency as patients with eating disorders show on matters relating to food intake and body image.

In a corollary series of studies in bulimic patients, we explored the secretion of osmotically mediated arginine vasopressin secretion and the secretion of arginine vasopressin into the CSF. In contrast to underweight anorexics, only a minority of normal weight bulimic subjects showed a complete disruption in the function of the hypothalamic osmoreceptor. However, as in the anorexic subjects (and to a greater extent), arginine vasopressin is markedly hypersecreted into the CSF. We are currently in the process of attempting to further define this subgroup with increased CSF vasopressin

levels and, to conduct studies in experimental animals following up on preliminary data suggesting that vasopressin may be a significant link between eating and drinking behavior (*vide infra*).

In keeping with our interest in the structurally related neurohormones, arginine vasopressin and oxytocin, which exert reciprocal effects on a variety of phenomena (e.g., learning and memory, ACTH release, etc.), we have shown that restrictor anorexics studied in the underweight phase show a marked decrease in the level of CSF oxytocin compared to either controls or both underweight and normal weight bulimic subjects. These data are of interest not only in terms of the fact that a decrease in the secretion of oxytocin into the CSF could enhance the defect of exaggerated CSF vasopressin secretion in anorexia nervosa, but also because recent data show that the firing rate of hypothalamic oxytocin neurons and the secretion of oxytocin is markedly enhanced during a variety of disparate situations known to terminate food intake (e.g., feeding, experimental gastric distension, peripheral infusion of cholecystokinin (acting at vagal stimulation)). Of potential significance in this study is that the only subgroup of patients with eating disorders shown to have abnormal secretion of oxytocin into CSF is that subgroup which consistently fails to stimulate satiety mechanisms (and hence, possible central oxytocin release) by the ingestion of significant amounts of food at some time during the course of the phase of illness under study.

Another implication of this study is that it represents the first biologic data to support the merging idea based on extensive clinical observations that restrictor anorexics represent a unique subgroup and that bulimic anorexic patients manifest an illness much closer to that manifested in normal weight bulimics.

In a further series of studies in anorexics, we have shown that the levels of CSF ACTH and beta-endorphin are markedly reduced in the underweight phase. In the light of strong positive correlations between CSF CRH and CSF ACTH in controls, it was unclear whether these data reflected depletion of central POMC stores by chronic hyperstimulation by CRH, or glucocorticoid inhibition of ACTH and beta-endorphin synthesis and/or release (i.e., patients with Cushing's disease also show a marked diminution in the levels of CSF ACTH). These data have stimulated our interest in the role of ACTH and beta-endorphin in parameters of interest to the study of eating disorders such as appetite regulation, sexual function and mood. In this regard, we have recently completed analyzing a series of clinical studies exploring the effects of naloxone on appetite regulation and neuroendocrine function in patients with anorexia nervosa and bulimia, indicating deficient inhibition of feeding and insufficient disinhibition of hypothalamic-pituitary-adrenal function in both populations.

As noted in the introduction of this section, one of the questions we wished to address was whether possible neural mechanisms subserving hunger and satiety may be preferentially abnormal in the eating disorders con-

ferring upon them the unique characteristics which distinguish them from the affective disorders. Our studies in oxytocin and vasopressin regulation have represented one effort in this area. A more direct series of studies aimed specifically at exploring mechanisms of satiety and hunger has been conducted by Geraciotti et al., exploring the potential role of cholecystokinin in the binging behavior in normal weight bulimics. Dr. Geraciotti demonstrated (N. Engl. J. Med., in press) that the plasma levels of cholecystokinin, which are thought to mediate satiety via an action on vagal receptors (and in laboratory animals results in the increased firing of oxytocinergic neurons) are markedly deficient during feeding in bulimics. This failure of cholecystokinin secretion during feeding in bulimics correlated with the lack of satiety experienced during the administration of a test meal as well as with the amount of food ingested. Moreover, this abnormality in cholecystokinin secretion and satiety during test meal administration resolved in patients with bulimia who responded to the administration of tricyclic antidepressants. These data represent the first suggestion that, in addition to depressive features, patients with bulimia may show a specific abnormality in mechanisms of satiety which contributes to the expression of their eating disorder.

In further studies exploring the secretion of peptides modulating food intake in clinical populations with the eating disorders, we have shown, in collaboration with Walter Kaye, that the secretion of neuropeptide YY, which markedly enhances food intake, is profoundly elevated in patients with bulimia upon admission to hospital. In fact, in each patient in the study, the level of PYY was entirely above the normal range. These data represent a second finding suggesting that patients with bulimia suffer from a neurohormonal defect which could contribute to an enhanced drive to ingest food and impaired mechanisms of satiety.

Studies of CCK regulation in restrictor and bulimic subjects have recently commenced. Although peptide YY levels were normal in a preliminary study of underweight patients with anorexia nervosa, underweight anorexics showed a significant increase in the level of neuropeptide Y (NPY), another peptide shown to enhance food intake in experimental animals. These data are compatible with the clinical observation that patients with anorexia nervosa often manifest a voracious appetite which is overridden by their obsessional preoccupation with the adverse consequences of eating. This clinical observation constitutes one of the reasons for a proposed trial of the anorexic agent fluoxetine we plan in anorexia nervosa. This antidepressant agent has been shown to be effective in controlling obsessional symptoms and by diminishing appetite in anorexic subjects may permit them greater peace of mind that they will not profoundly overeat if they relax their rigid controls upon food intake. Parenthetically, levels of NPY were also elevated in weight-recovered anorexics studied after the short-term correction of weight loss, and were highest in those who had failed to regain normal menstrual function. This finding is of interest in the light of data that NPY inhibits LH-RH secretion as well as the gonadotropin response to LH-RH. Hence, we postulate that NPY may contribute to the hypothalamic hypogonadism, to which

CRH (via centrally mediated beta-endorphin release and peripheral pituitary-adrenal activation) undoubtedly contributes.

Hypersecretion of growth hormone is a defect which is common to both major depression and anorexia nervosa. Our interest in this abnormality relates both to our wish to compare and contrast pathophysiological mechanisms in the affective and eating disorders and to the fact that GH-RH has behavioral as well as endocrine effects which consist of enhancement of food intake and sleep. In this regard, we postulate that GH-RH release during stress may represent an effort to counterregulate the arousal-anorexic effects of CRH.

To explore the pathophysiology of growth hormone hypersecretion in major depression and anorexia nervosa, we administered 1 ug/kg of GH-RH and measured GH and somatomedin C levels before and after the infusion. We noted that in patients with depression, GH responses to GH-RH were significantly attenuated in association with elevated levels of basal somatomedin C. In contrast, patients with anorexia nervosa showed exaggerated GH responses to GH-RH in association with a significant decrease in basal somatomedin C levels. We postulate that the blunted GH responses in depression reflect negative feedback inhibition of the pituitary somatotroph by elevated levels of somatomedin C. The elevation in somatomedin C strongly indicates that GH-RH is hypersecreted in depression to produce an increase in GH-mediated somatomedin C production. In contrast to depression, the GH response to exogenous GH-RH is disinhibited in anorexia nervosa as a consequence of the deficient levels and/or actions of somatomedin C. Such a deficiency in levels is likely a consequence both of hypercortisolism and inanition, while decreased biologic activity of somatomedin C has been shown to be a consequence of hypercortisolism. In contrast to our inference that GH hypersecretion in depression represents hypersecretion of endogenous GH-RH, the present data in anorexia nervosa do not permit such an inference. Hence, hypersecretion of GH could reflect disinhibition of the GH-RH neuron by somatomedin C deficiency or simply disinhibition of the somatotroph response to GH-RH because of the same defect. To further clarify this question, we are currently exploring the effects of food deprivation on GH-RH message expression in the arcuate nucleus via *in situ* hybridization.

Our interest in GH-RH in depression and anorexia nervosa is paralleled by our interest in somatostatin. In collaboration with Rubinow, we were among the first to show a significant decrease in the level of CSF somatostatin in patients with major depression. Our finding that CSF somatostatin is profoundly diminished in patients with Cushing's disease suggests that the decreased CSF somatostatin levels in depression are a consequence of hypercortisolism (i.e., we have shown that the hypercortisolism of Cushing's disease is a peripheral rather than a central defect). More direct evidence for the premise that hypercortisolism of depression is responsible for the decreased somatostatin levels in this disorder derives from the work of Wolkowitz, et al., who showed that prednisone administration to volunteers significantly dimin

ished the levels of somatostatin in the CSF.

In contrast to patients with major depression, CSF somatostatin levels in hypercortisolemic underweight patients with anorexia nervosa are similar to those seen in normal controls. Although we cannot definitively account for this finding, the significantly higher centrally directed CRH in anorexia nervosa compared to major depression could contribute to normalization of somatostatin secretion in anorexia nervosa. In this regard, several lines of data show that CRH stimulates the *in vivo* and *in vitro* secretion of somatostatin.

(B) Pre-clinical studies:

Progress: One of the goals of our preclinical studies has been to explore whether CRH and arginine vasopressin show synergy in brain analogous to their synergy at the pituitary corticotroph. To explore this, Dr. John Glowa is conducting a series of studies to determine if arginine vasopressin shifts the dose-response curve for icv CRH effects on motor function and lick suppression. In a less direct demonstration of interactions between these peptides, we have recently shown that arginine vasopressin stimulates CRH release from hypothalamic cell culture, an effect antagonized by specific vasopressin VI antagonists. Parenthetically, carbamazepine, an agent which enhances renal responsiveness to arginine vasopressin, also seems to enhance the responsiveness of the pituitary corticotroph cell to vasopressin, an effect blocked by the V1B vasopressin antagonist. We are currently in the process of assessing the effects of carbamazepine on arginine vasopressin receptors in brain and also on site-specific expression of the arginine vasopressin gene in brain via *in situ* hybridization.

To explore whether decreased CSF ACTH and beta-endorphin in anorexia nervosa reflects depletion of POMC by CRH or decrease in POMC synthesis, we conducted studies in food-deprived rats and estimated POMC message in the arcuate nucleus by *in situ* hybridization. This study demonstrated a marked diminution in POMC message in this locus. We are currently assessing whether the effect of food deprivation can be reversed utilizing pretreatment with a glucocorticoid antagonist. Additional studies in food-deprived rats show that CCK message is markedly depleted in the PVN of female but not male rats. We also see that female rats show a markedly greater degree of hypercortisolism during this stress and are currently awaiting analysis of CRH message content in male and female food-deprived rats. Additional studies utilizing probes for CCK, NPY, and PYY are also underway.

4. Studies of the circadian pattern of neuropeptide and neurotransmitter secretion into the CSF in clinical populations and in animal models, as a means of both further studying the pathophysiology of major psychiatric disorders as well as further exploring the endocrine functions of the brain and the mechanisms of parasynaptic information-processing.

Progress: A major tenet of both the basic and clinical studies of our group is that informational substances act at receptors in hormone-like fashion.

ion after release from nerve terminals in the CNS. All brain cells are bathed in interstitial fluid, which is CSF, also comprising the major ventricular cavities. Collectively, the CSF occupies 20% of the total volume of the brain. Every informational substance released from nerve terminals can be measured in CSF, and levels of CSF fluctuate with changes in neural activity. Hence, in both our clinical and basic studies, the physiology of neurotransmitter release into the CSF reflects an issue of intense theoretical and practical interest.

In the clinical realm, we have initiated a comprehensive study of the circadian organization of neurohormonal and neurotransmitter secretion into the CSF in human volunteers and patients with major psychiatric disturbances. At present, we have studied four volunteers and two patients during depression and following ECT-induced recovery. Preliminary data at present indicate that CRH and ACTH are both secreted into human CSF in a pulsatile fashion, and show a circadian variation which is the inverse of pituitary-adrenal rhythms. In depression, the amplitude of CSF CRH pulses are normal despite marked hypercortisolism, an abnormal finding in light of data that glucocorticoids inhibit secretion of CRH into the CSF. Following recovery, there is a highly significant fall in CSF CRH levels compared to depression. Levels in recovered subjects are also significantly lower than in controls, suggesting either some residual effects of glucocorticoid negative feedback analogous to that seen in Cushing's disease several months following surgical correction, or hyperresponsiveness of the adrenal cortex to ACTH with resetting of the CNS CRH system accordingly.

Dr. John Glowa, working with Drs. Miles Herkenham, Konstantine Kalogeris, and Linda Brady has set up a corollary model in rhesus monkeys, with indwelling cannulae in both the cerebral ventricles and the lumbar space. This model will help us to directly assess the issue of gradients with respect to human study as well as to further work out the regulation of peptide secretion into the CSF. We can also administer substances into the CSF space with this model. As a first study, immunoneutralization of CRH with CRH antisera may help us to demonstrate whether CRH in the CSF space or in periventricular regions (given the limited absorption of icv antisera) plays a role in either behavior or physiology. We shall also be able to assess the effects of icv-injected peptides on the expression of CRH and related genes in areas such as hypothalamus, locus ceruleus, and hippocampus via *in situ* hybridization.

One of the goals of our studies with the CSF in keeping with the theme of the endocrine functions of the brain has been to measure channels and rates of penetration of substances from the CSF into the brain parenchyma after administration into larger, ventricular cavities. As a first study, we compared the distribution of inulin to that of CRH. Both molecules are relatively large (MW 5,000), but inulin is an inert compound that stays in the fluid compartment, whereas CRH is a peptide susceptible to normal degradative processes. We found that both compounds distribute rapidly throughout the major cavities, from which they slowly penetrate the brain parenchyma. After several hours, we find that the entire brain is bathed in radioactive inulin,

whereas CRH is active for only about an hour before degradation, during which time it penetrates several millimeters into the brain, most notably into the hypothalamic and LC areas. Radioimmunoassay confirms that the radioactivity we observe is authentic CRH at each of the selected penetration times.

These observations serve as the backdrop against which we can pursue functional questions about the role of the CSF in CNS and neuroendocrine physiology. We have found by this technique that the locus ceruleus is a major source of norepinephrine into the CSF and that the superior cervical ganglion is not. Currently, we are examining the role that the locus ceruleus plays in the regulation and expression of message for CNS peptides. These studies, which are among the highest priority of those proposed in this area of investigation, highlight the close interrelationship between the neuroanatomic and molecular components of our laboratory and the close coordination between clinical and preclinical contingencies.

5. Interactions between hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid interactions in clinical populations (including major depression) and animal models.

Progress: The HPA and HPT axes represent the two neuroendocrine axes most prominently disturbed in patients with major affective illness. Although our work has focused on the CRH system and its relevance to pituitary-adrenal function in normal physiology and patient populations, we have also conducted a series of studies to explore the interactions between HPA and HPT axes. In the first of these studies, conducted in patients with clinically-manifest hyperthyroidism, we demonstrated that such patients show an exaggerated ACTH response to exogenous CRH more subtle but analogous to that seen in patients with adrenal insufficiency. To explore this further in animal studies, we now show that rats made hypothyroid show decreased CRH content in hypothalamus. Conversely, hypothyroid animals show increased hypothalamic CRH content. We are currently following up these findings with an extensive series of studies in hyper- and hypothyroid rats to explore the effect of these conditions on gene expression in disparate sites in brain via *in situ* hybridization, including, of course, the effect of these conditions on CRH gene expression in PVN, nucleus paragigantocellularis, and other sites including amygdaloid nucleus and cerebral cortex. In keeping with clinical data that patients with hypothyroidism have difficulty excreting a water load due to excessive secretion of arginine vasopressin, we have also begun to explore responsiveness to AVP in hypo- and hyperthyroid rats. A preliminary finding in this study is that hyperthyroid rats show extraordinary sensitivity to minute doses of vasopressin through as yet unidentified mechanisms.

A long-term project in collaboration with Dr. Cary Weinberger has been to explore the regulation of thyroid hormone receptor function in brain. The thyroid hormone receptor gene shows strong homology with glucocorticoid, mineralocorticoid, and progesterone receptors. A specific project undertaken by Dr. Mark Smith has been to attempt to isolate thyroid hormone

receptor genes in brain apart from the one already cloned by Dr. Weinberger. This project has been undertaken because evidence from Southern blots analyzed under non-stringent conditions suggests that a number of brain thyroid hormone receptors may exist. Dr. Smith is also working to explore the effects of hyper- and hypothyroidism on thyroid hormone receptors in brain via *in situ* hybridization as well as to assess the effects of glucocorticoid administration and glucocorticoid antagonism on this parameter.

6. Development of animal models of depression and anorexia nervosa and the study of physiological, behavioral, and molecular correlates.

Progress: For the past two years, we have maintained a colony of fifty marmosets in Building 14 under the supervision of full-time Guest Worker Elizabeth Johnson-McClure. Dr. Johnson-McClure has had extensive experience with this species (*Callitrix Jacchus*) and has been able to successfully breed them in Building 14, in contrast to efforts undertaken in Poolesville. Dr. Johnson-McClure has also developed a close relationship with these marmosets so that she is able to hold them in her arms and take blood for hormonal measurement in about thirty seconds without restraining them.

In the past year, Dr. Johnson-McClure has validated scales to rate young marmoset mother-child interactions based on their positive-negative interactions (e.g., carrying, social exploration, nursing, food sharing, proximity huddling, vs. bite, grabbing, rejection, attempt rejection, etc.). Dr. Johnson-McClure has been able to show that a quotient representing the sum of caring behaviors minus the punitive behaviors correlates positively with head circumference, limb length, and weight at six months. These data constitute the basis for the first naturalistically occurring model of psychosocial growth retardation. Follow-up of these marmosets at puberty shows that those who had relatively unsatisfactory interactions with the mother continue to show subtle but significant signs of growth retardation.

In follow-up studies, Dr. Johnson-McClure, in collaboration with other members of our group, has begun to study the response of those marmosets whom she has followed since birth to various behavioral situations. Having had extensive experience in evaluating the behavior of these pair-bonding primates, Dr. Johnson-McClure is currently studying them under several social conditions which are by definition differentially stressful. These conditions are in order of stressfulness, least to most: family, heterozygous pairs, social isolation, and peer group pairs (three males and three females). The following observations have been made. Wasting, a serious syndrome associated with behavioral depression and anorexia, occurs most often in female subordinates in the peer group. Isolated and subordinate animals show evidence of an inactivation of the CRH system (analogous, possibly to atypical depression) with decreased basal ACTH levels and increased ACTH responses to ovine CRH. Behaviors in isolation and in subordinates are very similar, often occurring in short spurts and characterized by many infantile behaviors like infant crying.

In addition to further behavioral and physiological studies (the colony is continually increasing) of deepening complexity, as numbers permit we will hopefully be able to draw inferences about the vulnerability to development of behavioral depression or wasting in the peer groups or isolates based on mother-child interactions as potential influential parameters. We also hope to determine whether the development of subordination is related to the tenor of mother-child interactions. In the 10% of animals who develop the wasting syndrome, we plan an intensive investigation of brain function early on before profound weight loss has occurred (once the subtlest signs of wasting occur, the syndrome is invariably fatal, though ECT reversible); these studies include integration of the various techniques available in our preclinical laboratory including high resolution autoradiography for receptor mapping, as well as assessment of gene expression for a variety of neuropeptides and other proteins of interest utilizing *in situ* hybridization. Ultimately, if the model develops sufficiently so that there are large numbers and families with inherent vulnerabilities to mother-child maladaptation or wasting, other molecular techniques including screening for RFLP's may be feasible.

7. Differential Diagnosis and Pathophysiology of Major Depression and Cushing's Disease:

One of the persistent problems in clinical neuroendocrinology has been determining the differential diagnosis between major depression and Cushing's disease. Indeed, because depression can present with hypercortisolism of the magnitude of that seen in Cushing's disease, while patients with Cushing's disease can present with major depression, the two entities can be impossible to distinguish from one another. On the basis of the overlapping clinical and biochemical symptomatology, some have suggested that the two entities share pathophysiological features. In his 1973 Sir Henry Dale Lecture, Grant Liddle termed the problem posed by the differential diagnosis and pathophysiology of major depression and Cushing's disease as one of five enduring endocrinologic enigmas.

We have conducted a series of studies to explore the differential pathophysiology and diagnosis of these two illnesses. The first of these involved exploring the functional integrity of the pituitary corticotroph cell by administering ovine CRH. In patients with depression, plasma ACTH's were attenuated in proportion to the degree of basal hypercortisolism, indicating that the pituitary corticotroph cell in this disorder was appropriately restrained by glucocorticoid negative feedback. On the other hand, patients with Cushing's disease showed exaggerated ACTH responses to ovine CRH despite basal hypercortisolism, indicating that the pituitary corticotroph cell was grossly unresponsive to the feedback effects of the glucocorticoids. These findings represented the first clinical data in which responses obtained in patients with depression and Cushing's disease went in the opposite direction, and the limited overlap in the responses rendered the ovine CRH test as an extremely useful one in ascertaining the differential diagnosis of these entities. To date, our data show less than 18% overlap between the two groups.

In addition to data suggesting differences in the functional integrity of the pituitary corticotroph in these disorders, we also showed that the hypothalamic CRH neuron also seemed differentially organized. In depression, in addition to the fact that the pituitary corticotroph seemed normally responsive, two other lines of data suggested that hypothalamic CRH was involved in the hypercortisolism of this disorder. First, in volunteers given a continuous infusion of CRH, the resultant pattern and magnitude of cortisol secretion was very similar to that seen in Cushing's disease. In addition, CSF CRH levels correlated positively with indices of pituitary-adrenal activation and, like cortisol secretion itself, were positively correlated with age in patients with major depression. On the other hand, two lines of evidence suggested that in patients with Cushing's disease the hypothalamic CRH neuron was appropriately restrained by hypercortisolism. First, in the post-operative period following the transsphenoidal removal of a microadenoma (when patients are usually adrenally insufficient), most all patients respond with a clear ACTH response to exogenous CRH. This suggests that if the patients' own endogenous CRH had not been suppressed by long-standing hypercortisolism, these patients with responsive pituitaries would not have manifested adrenal insufficiency. Second, we have shown that patients with Cushing's disease show a profound decrease in the level of CSF CRH, compatible with our pre-clinical data that the neuronal elements which secrete CRH into the CSF are glucocorticoid suppressible.

We have conducted additional studies to explore the CNS milieu of Cushing's disease and to further elucidate differential pathophysiologic features between Cushing's disease and depression. In addition, we have further attempted to refine means for the differential diagnosis of these entities. As an example, we have shown that while plasma ACTH levels in Cushing's disease is quite high, the level of ACTH in the CSF of patients with Cushing's disease is extremely low, and in the majority of cases falls below the normal range. The latter finding is compatible with our preclinical data showing that the neurons secreting ACTH into the CSF are profoundly glucocorticoid-suppressible. In contrast to patients with Cushing's disease, who show very high plasma ACTH values in association with very low CSF levels, patients with depression show CSF-ACTH levels which are almost always greater than plasma ACTH levels. Indeed, the overlap in terms of the CSF/plasma ACTH ratio in patients with depression and Cushing's disease is less than 5%. Hence, this measure provides the best available means for ascertaining the differential diagnosis of these two entities.

In additional studies of the CNS milieu in patients with Cushing's disease, we have measured the levels of somatostatin in the CSF. Somatostatin levels are profoundly reduced in the CSF of Cushing's patients. This finding in a patient population with no demonstrable primary CNS pathology suggests that the reduction of somatostatin in the CSF is a reflection of hypercortisolism, and is compatible with the idea that the reduced levels of somatostatin in the CSF of depressed patients shares a similar mechanism. An additional finding in patients with Cushing's disease is that CSF CRH and CSF somatostatin

are positively correlated. This finding is compatible with preclinical data that CRH is a potent stimulus to the release of somatostatin. Hence, one of the reasons that CSF somatostatin is considerably lower in the CSF of Cushing's disease patients than in depression is that not only are cortisol levels generally higher in Cushing's disease, but also glucocorticoid inhibition of CRH secretion may diminish the impact of a stimulus to somatostatin secretion.

On the basis of our data that the CRH neuron in Cushing's disease is profoundly suppressed by long-standing hypercortisolism while that in depression is responsive and hyperactive, we have devised additional protocols to elucidate their differential diagnosis based on this pathophysiological information. For instance, we have commenced a study involving the infusion of intravenous alprazolam. Our preclinical studies show that this agent is a potent inhibitor of the hypothalamic CRH neuron but has no direct effect upon the pituitary corticotroph cell. On the basis of these data, we predict that patients with Cushing's disease, whose hypothalamic CRH neuron is already profoundly depressed, will show no pituitary-adrenal response to CRH, while patients with depression, who have a responsive CRH neuron, will respond with considerable pituitary-adrenal suppression.

8. Studies relevant to post-partum and menstrually-related depressions:

Pregnancy is the only known state in humans in which CRH circulates in plasma at levels expected to cause activation of the pituitary-adrenal axis (100-4,000 pg/ml). These levels gradually increase with advancing pregnancy, with further elevations during labor. Interestingly, it has been known for years that pregnancy has been associated with plasma-free hypercortisolism of the magnitude seen in depression and anorexia nervosa. We speculate that the remission seen in patients with depression or panic disorder during pregnancy could relate to glucocorticoid suppression of CRH and/or locus ceruleus-norepinephrine systems. Similarly, we speculate that following delivery of the placenta, when there is abrupt resolution of pregnancy-associated hypercortisolism, there may be symptoms of steroid withdrawal, which could contribute to the post-partum blues phenomenon. On the other hand, the long-standing hypercortisolism of pregnancy could produce relatively sustained suppression of the CRH system, analogous to the suppression of the CRH neuron occurring in Cushing's disease but of a lesser magnitude. In this regard, if the CRH system were to reoccur within a month or two or to rebound at this time, this could be associated with or contribute to the post-partum depression syndrome, which interestingly does not supervene until one or two months or even later after delivery. To explore these possibilities, in collaboration with Dr. Douglas Rabin, the guest worker in our group trained as a reproductive endocrinologist, we have commenced a protocol exploring dynamics of hypothalamic-pituitary-adrenal function during pregnancy and at regular intervals after delivery.

In pre-clinical studies, we have concentrated our efforts on studies of the regulation of placental CRH secretion. To accomplish this task, we developed an *in vitro* perfusion system in which full thickness placental fragments are kept in culture. These fragments contain a 1300-nucleotide mRNA which hybridizes with a CRH-specific oligonucleotide probe and secretes large quantities of hCRH *in vitro*. The latter is chromatographically identical to rat CRH. Glucocorticoids, prostaglandins, catecholamines, oxytocin, and vasopressin have no inhibitory or stimulatory effects on placental secretion of CRH. Our perfused placental fragments also secrete immunoreactive beta-endorphin, alpha MSH, and minute amounts of ACTH, also chromatographically identical to the corresponding synthetic peptides. Both CRH and oxytocin markedly stimulate the secretion of beta-endorphin and alpha MSH by the placenta. Further work in the functional regulation of placental peptide production and secretion are currently in progress.

The pre-menstrual depression syndrome is now well-defined and clearly recognized as a diagnostic entity. We are approaching this illness in a focal way, exploring the potential role of CRH in this entity. Our conceptual approach rests in part upon the observation that progesterone has substantial interactions with the glucocorticoid receptor and serves as a functional glucocorticoid antagonist. In this regard, during luteal phase progesterone secretion with its attendant glucocorticoid blockade, the CRH neuron would have to function with a higher gain in order to maintain basal glucocorticoid effects, possibly exacerbating an underlying diathesis to affective disorder. To explore this possibility, we are examining the effects of progesterone on pituitary-adrenal responsiveness to stimuli such as CRH. In addition, we have completed a study exploring pituitary-adrenal responsiveness to CRH in healthy women studied sequentially during early follicular, early luteal, and late luteal phases. We have also commenced a similar study in patients with the pre-menstrual tension syndrome.

Significance to Biomedical Research and the Program of the Institute:

The Section on Clinical Neuroendocrinology has attempted to delineate through a series of coherently related clinical and pre-clinical studies the mechanisms of physical and emotional stress and their relevance to major psychiatric illness. Accordingly, our clinical studies focus on stress-responsive neuromodulators in stress-related illnesses such as major depression and anorexia nervosa, while pre-clinical studies explore the interactions of the CRH and locus-eruleus norepinephrine studies utilizing techniques such as high resolution autoradiography and *in situ* hybridization. In addition, we have attempted to develop an animal-model of stress-induced depression in the primate. Our recent focus on anorexia nervosa and bulimia has also led us to attempt further clinical and biochemical definition of anorexia nervosa and bulimia, to clarify their relationship to the affective disorders and, as a corollary, to consider possible neural mechanisms subserving hunger and satiety which may confer pathophysiological specificity upon these illnesses. Studies

of neuroendocrine disorders such as Cushing's disease have also been undertaken not only because of an interest in elucidating pathophysiological mechanisms but also because such neuroendocrine illnesses can be difficult to distinguish from psychiatric disorders and are frequently characterized by mood and appetite disturbances.

Our work with one of the major effectors of the stress response, namely corticotropin releasing hormone, illustrates, in part, our approach to these goals. In a series of over 40 papers in the *New England Journal of Medicine*, the *Journal of Clinical Investigation*, and the *Journal of Clinical Endocrinology and Metabolism* we accomplished the following : (1) advanced the first data that CRH was of physiological relevance to human pituitary-adrenal function; (2) established that stress-induced pituitary-adrenal activation in humans required factors other than CRH; (3) showed that the pituitary corticotroph required the priming effects of its hypothalamic releasing factor to function properly; (4) compared and contrasted the pharmacokinetic and biological properties of ovine and human CRH; (5) showed that ovine CRH was best suited for diagnostic testing while human CRH could be used clinically to explore pulsatile ACTH secretion; (6) performed dose-response studies for human and ovine CRH and administered them at different times of day to work out the conditions for a clinically applicable CRH stimulation test; (7) applied the CRH stimulation test to work out the differential diagnosis between major depression and Cushing's disease, Cushing's disease from ectopic ACTH secretion, and secondary from tertiary adrenal insufficiency; (8) elucidated pathophysiological mechanisms in the hypercortisolism of major depression and Cushing's disease and proved that these two illnesses represented distinct pathophysiological processes; (9) showed that the hypercortisolism of major depression and anorexia nervosa had a similar pathophysiological basis attributable to CRH which could, in turn, contribute to the common clinical and biochemical manifestations of these illnesses; (10) developed hypothetical models for the potential role of CRH in other psychiatric illnesses characterized by hypercortisolism, including panic disorder; (11) elucidated the role of serotonin, norepinephrine, acetylcholine, GABA, benzodiazepines, excitatory amino acids, and feedback regulators such as beta-endorphin, ACTH, and CRH itself on the regulation of hypothalamic CRH release. All efforts to replicate this work have so far been successful, and the fruits of this work have been translated into everyday clinical practice in centers all over the world. In addition, our hypotheses regarding the etiology of major psychiatric illnesses such as depression and anorexia nervosa are among the most intensively pursued areas of investigation in psychiatric research.

In recent unpublished work, we have delineated a model invoking the simultaneous dysregulation of the CRH and locus-coeruleus norepinephrine systems in both melancholic and atypical depression (to be published as a two-part series in the *New England Journal of Medicine*). Our current unpublished data regarding this model include the following several lines of pre-clinical data that support our clinical data (e.g., positive correlations between

CSF CRH and NE, etc.) indicating concomitant activation of the CRH and locus ceruleus in melancholic depression and their mutual excitatory effect upon one another. These include: (1) establishment of an excitatory role for norepinephrine upon hypothalamic CRH release; (2) demonstration that icv CRH administration to the rat markedly increases locus ceruleus glucose utilization (while causing an expected concomitant decrease in hippocampal glucose utilization), (3) selective uptake of CRH by the locus ceruleus after icv administration, and (4) concomitant increase in CRH and tyrosine hydroxylase mRNA's after experimentally-induced stress. We also showed in our studies in patients with Cushing's disease, whose depressions are often atypical, that there is a concomitant inactivation of the CRH and locus ceruleus norepinephrine systems, as evidenced by marked reductions in both CSF CRH and MHPG. That these two forms of depression may be functionally related is indicated by data that during the natural history of periodic depression many individuals have melancholic depressions during one phase and atypical depressions during another.

Our studies with CRH have also potentially clarified a mechanism of limbic kindling and a possible role for CRH in the periodic, exacerbating course of major depression. Hence, not only have we shown that CRH can produce limbic seizures which cross-sensitize with electrically kindled seizures, but also that procaine administration to volunteers produces a dose-dependent activation of pituitary-adrenal function and a dose-dependent activation of hypothalamic CRH release which can be blocked by prior administration of carbamazepine. Recently, we have noted that CRH antisera attenuates the development of electrically-kindled seizures.

Our attempts to further define the clinical syndrome of anorexia nervosa have led to elucidation of significant dissociative processes in this disorder. In addition, we have compared and contrasted anorexia nervosa and depression with respect to the pathophysiology of hypercortisolism, growth hormone hypersecretion, and abnormal regulation of plasma and cerebrospinal fluid vasopressin regulation. With respect to the latter peptide, we have suggested that the hypersecretion of centrally directed vasopressin may not only synergize with the defect in CRH regulation but may contribute to the perseverative preoccupation with weight and body image in anorexic patients.

In studies of appetite regulation in bulimia, we have shown that bulimic patients show a significant attenuation in the plasma CCK response to food ingestion. In light of the fact that plasma CCK is thought to mediate satiety via interactions with peripheral vagal afferents, this finding may account for our observation that bulimics fail to show normal satiety during feeding. Additional studies of the neural substrates of appetite regulation include the demonstration that patients with bulimia show a marked hypersecretion of peptide YY into the CSF, in which all points fell above the normal range. PYY has been shown to be the most potent stimulus to food intake yet isolated. Hence, this finding constitutes a second indication of abnormal appetitive drive in bulimics which may interact with a diathesis to affective illness to confer clinical

and biochemical specificity to this illness.

In studies of oxytocin regulation in patients with the eating disorders, we have shown that the CSF secretion of this peptide is markedly abnormal in restrictor but not bulimic underweight anorexics. Similar levels of this peptide were found in underweight bulimic anorexics and normal weight bulimics. These data are of interest in light of reciprocal effects between oxytocin and vasopressin function in the CNS and a growing body of data showing significant roles for these peptides in appetite regulation and satiety. Moreover, these data represent the first demonstration of the clinical notion that restrictor anorexics represent a distinct illness from bulimic anorexics, who much more closely resemble patients with normal weight bulimia biochemically and clinically.

To study the endocrine functions of the brain and further delineate pathophysiological mechanisms in depression and anorexia nervosa, we have begun to sample lumbar CSF continually for thirty hours in our patient populations. To date, we have shown that peptide secretion into the CSF is pulsatile, that interactions among peptides and neurotransmitters occur, that CSF peptides and neurotransmitters display discrete circadian rhythms, that their levels and pattern of secretion relate to peripheral neuroendocrine function, and that the level and organization of peptides such as CRH and vasopressin are abnormal in major depression.

We have demonstrated for the first time close functional linkage between hypothalamic-pituitary-thyroid function and stress effector systems such as the CRH system. Hence, we have shown that hypothyroidism is associated with evidence of decrease in CRH function, while the converse is true of hyperthyroidism. Interactions between the structurally-related glucocorticoid and brain thyroid hormone receptors are also currently the object of intense study. It is well known that abnormalities in hypothalamic pituitary-thyroid and adrenal function constitute cardinal pathophysiological alterations in depressive illness.

We have developed an animal model of depression/anorexia nervosa in the primate based on naturalistic observations of interactions between offspring and mothers. We developed a scale of maternal-child interactions based on the number of caring behaviors minus the number of punitive ones and showed that the caring quotient correlated positively with head circumference, limb length and weight at puberty. Upon further study of the offspring in either heterozygote pairing, family setting, large peer groups, or isolation, we noted that a depressive wasting syndrome developed preferentially in offspring who had not been well cared for. This developed predominantly either in animals housed in isolation or in those who became subordinate in the large peer group. Biochemical correlates of this depression include a pathological inactivation of hypothalamic-pituitary-adrenal function. In those wasted animals who did not recover, we are currently examining post-mortem brain utilizing techniques such as high resolution autoradiography and *in situ* hybridization. This model provides the opportunity to study naturalistic evolu-

tion of mother-offspring interactions, the effects of poor interactions on growth development, and the context-dependent development of subsequent syndromes reminiscent of depression and/or anorexia nervosa.

Future Directions:

Interactions between the CRH, locus ceruleus-norepinephrine systems and their modulation by glucocorticoids will remain an important interest in the coming year. In clinical studies, two major protocols include the measurement of the circadian organization of neurohormones and neurotransmitters in the CSF via the continuous sampling of CSF through an indwelling lumbar drain, and the assessment of norepinephrine spillover into arterial blood during the basal state and after challenge with emotional, physical, and pharmacological stimuli. Studies in the well state will be emphasized to explore the premise that patients with major depression hyperrespond to stressful stimuli (possibly through an abnormality in glucocorticoid-mediated induction of tyrosine hydroxylase or activation of hippocampal glucocorticoid receptor-mediated negative feedback upon the CRH neuron. An additional focus of our clinical studies will be to explore our hypothesis that patients with atypical depression show a pathological inactivation of the CRH and LC-NE systems, in keeping with our data in patients with Cushing's disease (who often manifest symptoms of atypical depression) showing a glucocorticoid-mediated profound suppression of CSF CRH and CSF MHPG. In addition to the strategies mentioned above, we shall be assessing the 36-hour pattern of plasma ACTH and cortisol secretion followed by an a.m. CRH stimulation test. We predict that atypically depressed patients will hyperrespond to CRH at this time because of diminished glucocorticoid restraint of the pituitary corticotroph cell.

In addition to the clinical protocols noted here, we also plan to administer a two-week trial of glucocorticoids to melancholic and atypically depressed patients. We predict that such treatment will ameliorate melancholic depression and exacerbate atypical depression. Similarly, we plan trials of the glucocorticoid antagonist RU 486 to patients with these disorders, predicting that melancholics will show intensification of symptoms on the glucocorticoid antagonist, while patients with atypical depression will show amelioration.

In pre-clinical studies in this area, we plan to emphasize studies of the regulation of the glucocorticoid receptor by various physiologic and pharmacologic stimuli. For instance, one hypothesis which we will test is that tricyclics may be effective in the treatment of melancholic depression by increasing glucocorticoid receptor mRNA in the locus ceruleus and/or hippocampus. We shall also explore the effects of glucocorticoid agonists on this parameter, as well as a variety of neurotransmitters and neurohormones. Additional studies currently planned include studies of the effects of CRH antagonists or antisera on the effects of stimuli known to influence LC firing or TH expression, and the effects of noradrenergic antagonists on CRH mRNA expression under a

variety of conditions.

The mineralocorticoid receptor shows great structural homology to the glucocorticoid receptor and is expressed in great concentrations in the hippocampus. It shows greater affinity for cortisol than does the glucocorticoid receptor and is postulated to mediate the fine-tuning effects of the glucocorticoids on the CRH system. To explore the physiologic role of the mineralocorticoid receptor, Dr. Harvey Whitfield has commenced a project to mutate the mineralocorticoid receptor to make it constitutively active or to bind hormone but fail to bind to the DNA-binding region. One or more copies of these respective genes would be inserted into transgenic mice and the molecular, physiologic and behavioral effects systematically assessed.

We have commenced a parallel series of studies in patients with bulimia and anorexia nervosa as those outlined above in depression. A particular focus will be on analogies between bulimia and atypical depression and melancholic depression and anorexia nervosa. The focus on the neural regulation of hunger and satiety will continue, with evaluation of basal and stimulated plasma and CSF CCK measured via bioassay, and continued investigation of the biological basis and clinical correlates of abnormalities in oxytocin, vasopressin, NPY, and PYY. This area of investigation will be enhanced by the addition of Dr. Marion Hetherington to our staff, who brings extensive experience in the clinical evaluation of various components of eating behavior and in the construction of relevant animal models.

A new emphasis this year will be on the elucidation of the evolution of biological changes as the process of the correction of weight loss proceeds in underweight anorexics. We shall test the hypothesis that some of these changes may provoke anxiety and hence constitute a clinically relevant resistance encountered by the anorexic patient on the way to recovery. In the underweight phase, anorexics show a marked hyperfunction of the CRH system, but the LC-NE system seems quiescent as a means of conserving calories. Conversely, during the process of weight gain, the CRH system turns off, but there is some activation of the NE-LC system, perhaps in part secondary to food-stimulated insulin secretion. The mechanism(s) of this dissociation of CRH and LC-NE function in anorexia nervosa, its functional significance, and its possible relevance to the resistance anorexics show to tricyclic antidepressants and MAO inhibitors will be questions receiving further study.

Patients with anorexia nervosa show strong family histories of major depression. Bulimics show a similar family history plus strong family histories for addictive and impulsive disorders as well. Over the year, the eating disorders program has followed several large pedigrees of either anorexia nervosa/depression or bulimia/addictive disorders. One of our main aims will be to be able to expand our molecular biology program to permit RFLP screening of these pedigrees with probes relevant to our hypothetical models.

We plan to continue our studies exploring the differential pathophysiol

ogy of depression and Cushing's disease and hope to develop further means of assisting in their differential diagnosis. On the basis of our data that the hypothalamic CRH neuron in depression is hyperactive and responsive, while that in Cushing's disease is markedly suppressed by long-standing hypercortisolism and unresponsive, we plan to administer alprazolam IV to these two populations. We have shown that this agent is a potent suppressor of the CRH neuron utilizing our hypothalamic organ culture system but that it fails to suppress *in vitro* ACTH release by cultured pituicytes. Hence, we expect depressed patients to show a marked suppression of plasma ACTH following alprazolam, while patients with Cushing's disease should show no response at all.

In the following year, we hope to continue to expand the primate model of depression/anorexia nervosa developed by Dr. Johnson-McClure and other members of our group. We now have bred a sufficient number of animals and have taken them through puberty to placement in various social contexts to begin to assess whether an early history of poor maternal-offspring interactions with associated growth retardation predisposes to the development of the wasting syndrome in isolated animals or in those who develop subordinate roles in housing with a large peer group.

Our two major generic goals in the following year are to continue the vigor of our clinical studies, especially those exploring the relationship between melancholic and/or atypical depression and anorexia nervosa and/or bulimia. In the laboratory, the testing of our hypothetical models and the generation of new ones depends upon the presence of a vigorous molecular biology contingency to work closely with our other basic scientists. Drs. Mark Smith and Harvey Whitfield have gotten off to an excellent start but will require additional space and technical help to realize their goals.

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COLLABORATORS

- Dr. J. Amsterdam, Research Psychiatrist, Hospital of the University of Pennsylvania
 Dr. P. Avgerinos, Visiting Fellow, Developmental Endocrinology Branch, NICHD
 Dr. J. Ballenger, Chairman, Department of Psychiatry and Behavioral Sciences, Medical
 University of South Carolina
 Dr. R. Bernardini, Visiting Fellow, Clinical Neuroendocrinology Branch , NIMH
 Dr. W. Berrettini, Staff Psychiatrist, Clinical Neurogenetics Branch, NIMH
 Dr. L. Brady, Senior Staff Psychologist, Unit on Functional Neuroanatomy, CNE, NIMH
 Dr. D. Brandon, Chemist, Developmental Endocrinology Branch, NICHD
 Dr. H. Brandt, Senior Assistant Surgeon, Unit on Eating Disorders, Section on Biomedical
 Psychiatry, Laboratory of Clinical Science, NIMH
 Dr. J. Calabrese, Research Psychiatrist, Cleveland Clinic Foundation
 Dr. G. Chrousos, Senior Investigator, Developmental Endocrinology Branch, NICHD
 Dr. G. Cutler, Sr., Senior Investigator, Developmental Endocrinology Branch, NICHD
 Dr. R. Coppola, Laboratory of Psychology and Psychopathology, NIMH
 Dr. A. Calogero, Guest Researcher, DEB, NICHD
 Dr. R. Cowdry, Executive Assistant to the Director, NIMH
 Dr. C. Davis, Chemist, Biological Psychiatry Branch, NIMH
 Dr. M. Demitrack, NSRA Fellow, Clinical Neuroendocrinology Branch, NIMH
 Dr. P. Deuster, Assistant Professor, Department of Military Medicine, USUHS, United
 Services University of the Health Sciences
 Dr. J. Doppman, Chief, Department of Radiology, NIMH
 Dr. L. Dorn, Graduate Student, Department of Psychiatry, University of Pennsylvania
 Dr. P. Feuillan, Guest Researcher, Developmental Endocrinology Branch, NICHD
 Dr. D. Gardner, Clinical Neuroscience Branch, NIMH
 Dr. N. Garrick, Biologist, Section on Clinical Neuropharmacology, Laboratory of Clinical
 Science, NIMH
 Dr. T. Geraciotti, Medical Staff Fellow, Clinical Neuroendocrinology Branch, NIMH
 Dr. C. Gerfen, Senior Staff Fellow, Lab of Cell Biology, NIMH
 Dr. J. Glowa, Research Psychologist, Unit on Functional Anatomy, Clinical
 Neuroendocrinology Branch , NIMH
 Dr. F. Goodwin, Administrator, ADAMHA

- Dr. M. Grino, Guest Worker, Developmental Endocrinology Branch, NICHD
- Dr. A. Gulledge, Cleveland Clinic Foundation (Coral Springs, Florida Office)
- Dr. H. Gwirtsman, Department of Psychiatry UCLA School of Medicine
- Dr. M. Herkenham, Research Psychologist, Unit on Functional Anatomy, Clinical Neuroendocrinology Branch, NIMH
- Dr. C. Hoban, Psychologist, Unit on Peptide Studies, Biological Psychiatry Branch, NIMH
- Dr. M. Heatherington, Visiting Fellow, Clinical Neuroendocrinology Branch, NIMH
- Dr. E. Johnson-McClure, Guest Researcher, Clinical Neuroendocrinology Branch, NIMH
- Dr. J-P. Kahn, Hopital Jeanne d'Arc, Toul, France, and Biological Psychiatry Branch, NIMH
- Dr. K. Kalogeras, Visiting Fellow, Clinical Neuroendocrinology Branch, NIMH
- Dr. W. Kaye, Department of Psychiatry, University of Pittsburgh School of Medicine
- Dr. S. Kawai, Guest Researcher, Developmental Endocrinology Branch, NICHD
- Dr. C. Kellner, Research Psychiatrist, Medical University of South Carolina
- Dr. M. Kling, Senior Staff Fellow, Clinical Neuroendocrinology Section, Biological Psychiatry Branch
- Dr. T. Kuribayashi, Guest Researcher, Developmental Endocrinology Branch, NICHD
- Developmental Endocrinology Branch, NICHD
- Dr. L. Laue, Senior Staff Fellow, Developmental Endocrinology Branch, NICHD
- Dr. M. Linnoila, Clinical Director, Division of Intramural Clinical Biological Research, NIAAA
- Dr. G. Liddle, Cell Biology Laboratory, Mt. Zion Hospital Medical Center, San Francisco, CA
- Dr. D. Loriaux, Chief, Developmental Endocrinology Laboratory, NICHD
- Dr. A. Lugar, Visiting Fellow, Developmental Endocrinology Branch, NICHD
- Dr. M. Minichiello, Psychologist, Laboratory of Clinical Science, NIMH
- Dr. A. Margioris, Guest Worker, Developmental Endocrinology Branch, NICHD
- Dr. L. Nieman, Medical Staff Fellow, Developmental Endocrinology Branch, NICHD
- Dr. E. Nettleman, Developmental Psychiatry Laboratory, NIMH
- Dr. E. Oldfield, Chief, Surgical Neurology Branch, NIMCDS
- Dr. D. Pickar, Clinical Neuroscience Branch, NIMH
- Dr. T. Pigott, Staff Fellow, Laboratory of Clinical Science, NIMH
- Dr. R. Post, Chief, Biological Psychiatry Branch, NIMH

Dr. F. Putnam, Laboratory of Developmental Psychology, NIMH
Dr. D. Rabin, Guest Researcher, Clinical Neuroendocrinology Branch, NIMH
Dr. D. Renquist, Chief, Animal Center Section, Veterinary Resources Branch, NIH
Dr. G. Robertson, University of Chicago School of Medicine.
Dr. A. Roy, Medical Staff Fellow, Clinical Neuroscience Branch, NIMH
Dr. D. Rubinow, Clinical Director, NIMH
Dr. T. Schuermeyer, Postdoctoral Fellow, Deutsche Forschungsgemeinschaft, Essen, Federal Republic of Germany
Dr. H. Schulte, Postdoctoral Fellow, Deutsche Forschungsgemeinschaft, Essen, Federal Republic of Germany
Dr. M. Smith, Pratt Fellow, Unit on Functional Anatomy, Clinical Neuroendocrinology Branch, NIMH
Dr. B. Strupp, Division of Nutritional Sciences, Cornell University
Dr. E. Susman, College of Human Development, Pennsylvania State University
Mr. T. Tomai, Research Associate, Georgetown University Hospital
Dr. R. Udelsman, Department of Surgery, Johns Hopkins Hospital
Dr. H. Weingartner, Department of Psychology, George Washington University
Dr. C. Weinberger, Senior Staff Fellow, Laboratory of Cell Biology, NIMH
Dr. A. Winokur, Department of Psychiatry, University of Pennsylvania Hospital
Dr. H. Whitfield, Medical Staff Fellow, Clinical Neuroendocrinology Branch, NIMH
Dr. O. Wolkowitz, Assistant Professor, Department of Psychiatry, University of California San Francisco
Dr. S. Young, Senior Staff Fellow, Laboratory of Cell Biology, NIMH

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02433-01 CNE

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Central Nervous System Functional Anatomy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Miles Herkenham	Research Psychologist	UFN, CNE, NIMH
Others: Dr. L. Brady	Senior Staff Fellow	UFN, CNE, NIMH
Dr. J. R. Glowa	Research Psychologist	UFN, CNE, NIMH
Mr. M. Little	Biologist	UFN, CNE, NIMH
Dr. E. Mamalaki	Fogerty Fellow	UFN, CNE, NIMH
Dr. E. Richfield	NRSA Fellow	UFN, CNE, NIMH
Dr. R. B. Rothman	Staff Fellow	LCS, NIMH
Dr. C. Weinberger	Senior Staff Fellow	LCB, NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH; Laboratory of Chemistry, NIADDKD

LAB/BRANCH

Clinical Neuroendocrinology Branch

SECTION

Unit on Functional Neuroanatomy

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

10.0

PROFESSIONAL:

9.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our work can be divided into six major categories: neuroanatomy, neuropharmacology, neuroendocrinology, molecular biology, physiology, biology, physiology, physiology, and behavior. Mapping receptor localizations using the in vitro binding and autoradiography techniques yields important basic information about brain organization and function. The molecular biology group examines the regulation of specific genes for neuropeptides and monoamine synthesizing enzymes which have been implicated from clinical studies in the pathophysiology of psychiatric disorders and provides hypotheses based on findings from animal models which could be tested in patients. Similarly, the endocrinology effort addresses animal models of stress and the roles of peripheral hormones in CNS function. Physiological studies have examined regulation of opiate receptors in the neurohypophysis. Behavioral studies are designed to assess a variety of factors associated with anti-anxiety drug withdrawal, determinants of drug response, Maudsley rats, taste aversion learning, kindling, stress-related CNS changes, and central administration and monitoring of neuroendocrine factors in primates.

Objectives:

We seek to identify and quantify the regulation and expression of "informational substances" and their receptors in the brain. An understanding of receptor function requires knowledge of the biochemistry and pharmacology as well as the neuroanatomical localization of receptors. Specific, high-affinity, radiolabeled compounds are prepared by Dr. K.C. Rice, LC, NIADDKD. Receptors are identified by pharmacological criteria in collaborative studies with Dr. R. B. Rothman, LCS, NIMH. Probes for the genetic expression of mRNA, developed in collaboration with Dr. Cary Weinberger, Laboratory of Cell Biology, NIMH, or kindly provided by Dr. Scott Young III of the LCB, NIMH, are hybridized to brain tissue in combination with anatomical techniques to show the dynamic relationships between informational substances and their receptors, focusing on the hypothalamic-pituitary-adrenal (HPA) and opiate systems. We have chosen these systems because of the well-characterized roles of 1) corticotropin releasing hormone (CRH) and several other neuropeptides in the paraventricular nucleus of the hypothalamus and 2) opiates as central regulators which elicit a coordinated and coherent series of adaptive responses to stressful or painful stimuli.

Methods Employed:

We have successfully developed an in vitro autoradiographic technique for visualizing drug and neurotransmitter receptors in slide-mounted tissue sections. The details of this technique were described previously (Project number Z01 MH 01090-09 LNP). Radio-labeled tracer substances can also be mapped autoradiographically after systemic or intracerebroventricular injections in order to visualize distribution channels or target sites for biologically active drugs. Chronic drug delivery is made possible by surgical implantation of osmotic minipumps and (if necessary for delivering neuropeptides which do not cross the blood-brain barrier) ventricular cannulae. In vivo receptor autoradiography allows competition for binding between injected ligand and endogenous ligand, to enable us to make inferences about the status of endogenous systems during physiological manipulations. Dynamic activities in specified neurotransmitter systems are probed by the technique of in situ hybridization. Radioimmunoassay of plasma and cerebrospinal fluid (CSF) detects and quantifies levels of informational substances. Facilities for precise behavioral control and measurement have been set up to permit study of animal models of stress and mental disorders.

Major Findings:

Neuroanatomical studies. Mapping receptor localizations using the in vitro binding and autoradiography techniques, developed nine years ago by Herkenham and Pert, continues to yield important basic information about brain organization and function. In the opiate field, binding conditions for opiate receptor subtypes have been optimized so that studies of pharmacological and physiological mani-

pulations can be carried out in several appropriate species (rat, guinea pig, squirrel, marmoset, rhesus monkey). In a similar strategy, recently developed selective ligands for dopamine uptake sites are pharmacologically characterized.

Our work in the opiate and tachykinin systems and our use of comparative anatomy has provided the bulk of the supporting evidence for parasynaptic communication as a plausible explanation for the observation of mismatches between the locations of transmitters and receptors in brain. We have begun an aggressive program of functional approaches to an understanding of parasynaptic function in brain by combining physiological techniques with neuroanatomy.

Physiological studies. The rat neural lobe contains a single opiate receptor type, the kappa receptor, which is localized nonsynaptically on nerve terminals and pituicytes. Dynorphin-A and its fragments, which bind preferentially to the kappa receptor, are the predominant forms of opioid peptides in the neural lobe, and are co-localized with vasopressin in neurosecretory vesicles. Dehydration is a potent stimulus of the hypothalamic-neurohypophyseal system, causing the simultaneous release of vasopressin, oxytocin, dynorphins and enkephalins from neural lobe vesicles. Since kappa opiate receptor agonists produce a marked diuresis in rats presumably via an inhibition of vasopressin release, we have hypothesized that elevated release of opioid peptides during chronic dehydration is part of a biological mechanism to counter-regulate vasopressin release and conserve water. We examined the kinetic characteristics of kappa opiate receptors in the neural lobe of water-deprived and saline-treated rats and in homozygous Brattleboro rats using quantitative autoradiographic analysis. The data provided evidence for the *in vivo* down-regulation of opiate receptors by chronic osmotic stimulation. These findings support our hypothesis that kappa receptor down-regulation in the neurohypophysis occurs as a result of elevated co-release of vasopressin and opioid peptides induced by chronic osmotic stimulation, and functions to counter-regulate vasopressin release and conserve body fluids.

Neuroendocrine studies. In a different approach, we are focusing on CRH and its role in the production of a coordinated series of centrally mediated events collectively termed the stress response. Projects are under way to examine this response in rats, marmosets, and rhesus monkeys. Each species offers potentially important insights into the stress response and concomitant depression in humans. In one set of experiments rats are stressed acutely or chronically using footshock, restraint, or food deprivation. Plasma is taken for radioimmunoassay of corticosterone and ACTH levels as objective measures of stress. Brains are removed fresh and frozen for cryostat sectioning at several brain levels, notably the hypothalamus, at the level of the paraventricular nucleus, and the locus coeruleus. Genetic probes are generously provided by Dr. Scott Young of Dr. Michael Brownstein's Laboratory of Cell Biology, NIMH.

These are radiolabeled with ^{35}S for in situ hybridization on sections and subsequent autoradiographic visualization and quantification of mRNA levels of informational substances affected by the acute and chronic stressors. Currently we are examining message levels of vasopressin (AVP), oxytocin (OXY), corticotropin releasing hormone (CRH), cholecystokinin (CCK), neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), alpha melanocyte stimulating hormone (aMSH), somatostatin, thyroid hormone releasing hormone (TRH), thyroid stimulating hormone (TSH), tyrosine hydroxylase (TH), pro-opiomelanocortin (POMC), dynorphin (DYN) and enkephalin (ENK). Preliminary results are already quite fascinating. We have found that individual stressors produce specific and unique alterations in levels of expression of peptides and TH, suggesting characteristic modifications in brain neurochemistry depending on the type and duration of stressor. Additionally, because so little is known about the distributions of neurons that express and synthesize these substances, some brains have been sectioned for reconstruction and mapping of normal patterns. Thus, we are currently in a massive data-gathering phase that will have many long-term benefits.

Another excellent animal model of stress is the syndrome created in marmosets by physical separation of pair-bonded mates, siblings, or parents and offspring. These small primates respond to separation by showing a constellation of quantifiable behaviors which can be construed as manifestations of depression. The extreme form is called the "wasting syndrome." A battery of mRNA probes generated against primate (human) genes coding for many of the above-listed informational substances will be applied to brain sections of separated and normal siblings. In vitro receptor autoradiography will be carried out to determine whether and where changes in levels of opiate, CRH, and OXY receptors have occurred.

Rhesus intracerebroventricular (i.c.v.) studies are designed to develop a system for i.c.v. administration and central monitoring of neuroendocrine factors in monkeys. Surgery, in collaboration with Dr. John Bacher of the Veterinary Resources Branch, follows a unique approach that has not previously been successfully applied in the intramural program. With this system we are able to infuse drugs and peptides directly into the brain ventricular space and, with additional modifications, will be able to monitor CSF directly.

Molecular biology. In the field of molecular biology, the effects of thyroid and steroid hormones on brain gene expression are being studied. It is well known that hypothyroidism and hypercortisolism are associated with depression in humans. However, almost nothing is known concerning the effects of these hormones on adult brain function. We are in the middle of an extensive study of the effects of long-term hypo- and hyperthyroidism on neuropeptide and catecholamine function in the rat brain under basal conditions and stress. Using in situ hybridization we hope to determine which genes in the CNS are influenced by thyroid hormones, glucocorticoids, and miner-

alocorticoids.

In conjunction with Dr. Weinberger, we are screening a genomic library for thyroid hormone receptor genes. Dr. Weinberger has recently characterized a neural-specific thyroid hormone receptor which may be important in psychiatric disorders such as depression. Evidence from Southern blots suggests that additional thyroid hormone receptors exist.

Behavioral studies. Alprazolam withdrawal. This project is designed to assess a variety of factors associated with anti-anxiety drug withdrawal, as this effect remains a major drawback of prescribing anti-anxiety medication. Initial studies will focus on behavioral and neuroendocrine responses. Preliminary results have shown a rapid induction of tolerance to the disruptive effects of alprazolam on responding maintained by food presentation in rhesus monkeys. With as few as two subsequent administrations of alprazolam, tolerance to the disruptive effect can be demonstrated to 10-fold higher doses. These data suggest that changes in anxiolytic efficacy as well as changes in independent behavioral measures of disruption of responding relate to CNS changes associated with the development of therapeutic efficacy.

Maudsley rats. This project is intended to develop a line of rats which had previously been selectively bred based on an anxiety-like response in open field test and relate a number of biochemical, and molecular variables to their unusual behavioral traits. Preliminary data suggest that these animals are uniquely sensitive to environmental stimuli and do not exhibit typical anxiolytic response in drug tests such as the Vogel. Thus, initial work is designed to differentially breed these rats according to criterion previously established, and then use them in various models of stress and anti-anxiety drug effect. To date we have developed a colony and have behavioral scores on a sufficient number to proceed with both biochemical testing and differential breeding.

Taste aversion learning. Conditioned taste aversions are being studied in order to develop a better understanding of factors related to aversive properties of food-related events so that an animal model of reduced food intake may potentially be established; this would allow us to study neuroanatomical sites related to "psychologically" decreased food intake. We have treated animals with lithium chloride and found that central administration of several drugs and peptides appears not to produce a reduction in food intake in this model. Such results may allow us to narrow down the sites of action related to anorexic properties of drugs and peptides.

Kindling. In preliminary studies we have found that CRH release may mediate some effects of cocaine-induced kindling. In these studies the central administration of a CRH antibody blocked the development of kindling and associated lethality without blocking the behavioral

sensitization to repeated cocaine injections. We are attempting to replicate these findings and better establish the role of endogenous CRH in cocaine-induced seizures.

Stress-related CNS changes. Our studies of the effects of acute and chronic stressors on neuropeptide gene expression have been described above. In other studies, diethyl ether is used as a neuroendocrine stressor. Mice are exposed to a range of concentrations of ether. When operant responding is maintained, short exposures to low concentrations of ether produce large increases in responding, whereas higher concentrations abolish responding. The imidazobenzodiazepine Ro 15-4513 decreases responding, but only at doses of 10-30 mg/kg, while lower, non-behaviorally active, doses (3 mg/kg) antagonize the increases in responding produced by ether. These results suggest that some of the behavioral effects of volatile organic anesthetics may be mediated through GABAergic actions.

Significance to Biomedical Research and to the Program of the Institute:

The visualization by autoradiographic techniques of opiate receptor locations throughout the CNS has greatly advanced our appreciation of the richness of opiate functions in normal physiology and has led to new insights into receptor-mediated brain processes. Receptors that are not located at sites of synaptically released transmitter may be mediating transmitter action of a more hormonal nature. This parasynaptic or endocrine organization of the brain has many implications for biological psychiatry. For example, centrally acting drugs exert pervasive effects after diffusion through extracellular spaces and, thus, may mimic the mode of action of endogenous neurochemicals far more than was previously thought. Further work examining parasynaptic mechanisms should help to elucidate pathophysiological mechanisms in psychiatric illness and provide a foundation for better understanding of information transfer and consolidation in the brain. When these phenomena are understood in better detail, significant improvements can be expected in the approach to treatment of neuropsychiatric disease.

The endocrinology focus is a recently expanded research effort, with numerous approaches ongoing simultaneously. This effort represents a major bridge between the basic research of the UFN and the clinical studies of the CNE. Several studies address the common interests directly, such as our work on animal models of stress and the roles of peripheral hormones in CNS function. These studies are aimed at a better understanding of the roles that particular neuropeptides and monoamines play in the disorders created by stressful conditions.

In a planned set of experiments in rhesus monkeys, CRH, CRH antagonist, CRH receptor toxin, and other biologically active sub-

stances will be administered via implanted cannulae into the CSF of the lateral ventricles, and CSF and plasma will be collected to examine the relationship between CSF and plasma parameters. These data will be relevant to the interpretation of similar kinds of collections performed in normal and depressed patients.

Proposed Course of the Project:

With our growing appreciation that many receptors are nonsynaptically located and, therefore, may mediate parasynaptic intercellular communication in an endocrine fashion, we will explore several lines of pertinent inquiry. We propose to trace the movement of inert as well as biologically active peptides through the cerebrospinal and extracellular fluids. Other neuroanatomical work will use comparative and developmental approaches in several neurotransmitter/receptor systems (we will focus on the opiate and CRH systems) to gain insights into the rules of organization of the respective distributions. Correlative studies of the distributions of relevant molecules, such as synthesizing and degradative enzymes, and neural pathways identified by histochemical and physiological means will serve to generate hypotheses about functional operations within the systems. Quantitative autoradiographic techniques will be used to show dynamic relationships between transmitters and parasynaptic receptors. Molecular probes and in situ hybridization will be used to show the locations and physiological regulation of cells expressing the relevant markers. Similar studies carried out in human tissues will allow inferences to be made about the pathophysiology of psychiatric illnesses.

We are also investigating the functional roles of selected neuropeptides as components of central neuroendocrine systems. We plan studies in which transmitter synthesis, transport and release and receptor regulation are concurrently studied under controlled behavioral or physiological manipulations. As a result of such studies, it may be possible to develop animal models of psychiatric disorders, such as depression, with known endocrine abnormalities detected by aberrant fluctuations of peptide hormones in the cerebrospinal fluid or individual brain loci. Our choice of primates for many of these studies is based on the availability of large volumes of CSF for sampling studies (rhesus monkeys), on the ability to take advantage of complex social behaviors (pair bonding in marmosets), and the applicability of the results to similar human conditions.

Using in situ hybridization, we have found discrete changes in neuropeptide mRNAs such as corticotropin-releasing hormone (CRH) in the brains of rats subjected to various acute stressors. At present, we are examining the effects of chronic stress to determine how long the stress-induced changes in gene expression persist. We will also correlate these molecular changes with behavior of the rats and plan to do further research on the Maudsley strain which is supersensitive to stress. We are pursuing the interdependence of neuro-

peptide genes with neurotransmitters such as norepinephrine (NE). These studies will help test our hypotheses concerning abnormalities in CRH and NE which may be important in the pathophysiology of depression.

A long-term project in conjunction with Dr. Cary Weinberger involves the use of differential hybridization to identify steroid and thyroid hormone responsive genes in the rat brain. Adrenalectomized rats will be replaced with glucocorticoids or mineralocorticoids and then sacrificed several hours later to determine what genes are rapidly induced by these hormones. We have already made a rat hippocampal cDNA library in preparation for screening these hormone-induced genes.

One set of molecular biological experiments will examine the role of glucocorticoid receptors in the chronic stress response, using transgenic mice containing mutant mineralocorticoid receptors. As a long term goal we want to screen a genomic library of human brain for sequences which would hybridize with 5' and 3' flanking sequences in order to use those sequences to provide tissue appropriate expression of the human mineralocorticoid receptor in transgenic mice.

For the glucocorticoid receptor, we are working to make mutants by putting insertions which will create extra amino acids or mutants that are truncated. We will then link this modified mineralocorticoid receptor cDNA into SV 40-plasmid expression vector. A non-sense codon is introduced into the cDNA of the cloned receptor, and this blocks protein synthesis at the point in the messenger RNAs where any of those codons are present. We will then determine what genes are turned on when mineralocorticoids bind.

We will use the transgenic mice in studies of basic physiology, such as circadian rhythms of steroids and the pathogenesis of hypercortisolism. We will not only produce mice with constitutatively active receptors but also transgenic mice with differential number of receptor mRNA copies.

In another approach we will extract mRNA from hippocampus of rats treated with various steroids after adrenalectomy. A radioactive cDNA is used as a probe. All the mRNA's from hippocampus are labeled, introduced into the hippocampal library, and hopefully we will see some clones that are stronger in some animals than others. This technique is called differential hybridization, a truly shotgun method, but much easier than differential cloning.

In conjunction with Dr. Rabin, Developmental Endocrinology Branch, NICHD, we are looking at the effects of sex steroids such as estradiol on glucocorticoid and mineralocorticoid receptors and their genes in the rat hippocampus and pituitary. This may have important implications in our understanding of sex differences in

such illnesses as anorexia nervosa.

We wish to determine the changes in brain biochemistry which are a concomitant of weight loss in animals and compare these changes to what may be occurring in patients with anorexia nervosa. We have food-deprived rats and found specific changes in hypothalamic mRNA levels for peptides important in appetite regulation. In addition we are examining the "normal" neuroendocrine responses to reduced caloric intake in rats, some of which are the same but others quite different from what we have observed in anorexic patients. We can now begin to examine what controls these changes in hypothalamic peptide secretion and gene expression during food deprivation. Interestingly, differences occurred in the neuroendocrine response to food deprivation in male and female rats which may be important in our understanding of anorexia nervosa, an illness which afflicts females much more often than males.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00450-14 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Rhythms in Affective Illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Sack	Chief, Inpatient Services	CPB/NIMH
Others:	W. Mendelson	Chief, Unit on Sleep Studies	CPB/NIMH
	W. Duncan	Research Psychologist	CPB/NIMH
	N. Rosenthal	Chief, Outpatient Services	CPB/NIMH
	R. Skwerer	Medical Staff Fellow	CPB/NIMH
	F. Jacobsen	Medical Staff Fellow	CPB/NIMH
	T. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was terminated under this number but work continues under new project number: Z01 MH 02424-02 CP.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02197-02 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of the Effects of Light and Triazolam on Delayed Sleep Phase Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. Rosenthal, Chief, Outpatient Services CPB/NIMH

Others: K. Kelly Medical Staff Fellow CPB/NIMH

J. Vanderpool Medical Staff Fellow CPB/NIMH

P. Schulz Social Worker CPB/NIMH

T. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

E. Souetre Visiting Fellow CPB/NIMH

COOPERATING UNITS (if any)

Richard Allen, Ph.D., Johns Hopkins Sleep Center, Upjohn Pharmaceutical Company

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

0.5

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Delayed sleep phase syndrome (DSPS) is characterized by a chronic inability to fall asleep in the evening and to wake up refreshed early in the morning. These patients have an abnormally delayed sleep schedule in spite of normal sleep architecture. The goal of this study is to investigate the possibility of treating DSPS by manipulating the light-dark cycle. Patients were included in the protocol on the basis of clinical screening and polysomnography. The treatment involved a crossover study between two light-dark regimens: 1) 2 weeks of bright light (full spectrum, 2500 lux) from 7:00 a.m. to 9:00 a.m. and dark goggles from 4:00 p.m. to nightfall, and 2) 2 weeks of dim light (300 lux) from 7:00 a.m. to 9:00 a.m. and clear goggles from 4:00 p.m. to nightfall. Of nine patients treated under both conditions, the bright light-dark goggle condition has proven superior to the control treatment, as measured by self-report and morning sleep latency studies. Several of these patients have chosen to continue bright light treatment after the conclusion of the formal protocol. These findings suggest that judiciously timed bright and dark exposures are effective in advancing the circadian cycle of patients with DSPS.

Project Description and Methods:

A group of patients, who have difficulty going to sleep and waking up at conventional times, has previously been described. This condition, termed delayed sleep phase syndrome (DSPS), has been shown to cause significant unhappiness in some individuals, who are unable to get to work on time and suffer the social consequences of being on a different sleep-wake schedule from most other people. Typically these individuals have made many unsuccessful attempts to correct their abnormal sleep-wake cycle; indeed, it often appears to be rigidly set in place. A non-pharmacological treatment, called chronotherapy, in which the patient is advised to go to sleep at progressively later times each day until the desired sleep onset is reached, has proven successful in some individuals. However, it is an inconvenient treatment, involving several days of disrupted schedules and the benefits appear to be short-lived in many cases. Recent evidence suggests that alteration of the light-dark cycle can alter the timing of human circadian rhythms. In this study we have attempted to use properly timed bright light and dark exposures to shift sleep times in DSPS patients. We have also retained the option of using the short-acting benzodiazepine, triazolam, in conjunction with light-dark manipulations since this drug has been shown to shift the timing of free running biological rhythms in rats.

We recruited a population of patients with DSPS who were motivated to participate in a research study. Demographics were derived through the use of a specially designed DSPS screening questionnaire which was evaluated by a psychiatrist. Those who were determined to have DSPS were given a clinical interview and a SCID to confirm the diagnosis and to screen for active psychopathology which might contribute to abnormal sleep patterns. Subjects who passed this preliminary screening were asked to wear an ambulatory activity monitor and to fill out sleep logs for two weeks. After confirmation of DSPS by sleep logs and activity monitoring, patients were given a polysomnogram and a full physical to rule out other sleep disorders or major medical problems.

Subjects were treated in a crossover design in which conditions of light and dark were manipulated. They were assigned to two two-week treatments in random order: 1) two hours of bright (2500 lux) full-spectrum light in the morning from 7:00 to 9:00 a.m. in conjunction with dark goggles from 4:00 to nightfall; and 2) two hours of dim (300 lux) light in the morning from 7:00 to 9:00 a.m. in conjunction with transparent goggles from 4:00 to nightfall. We predicted that the former condition would be active and would shift the sleep-wake cycle earlier; the latter condition we hypothesized would serve as a control. Response to the interventions was evaluated clinically as well as by EEG-recorded sleep studies, multiple sleep latencies, 24 hour core temperature recordings, self-rated alertness, and sleep logs, as well as activity monitoring.

Findings to Date:

One hundred and seventy-eight subjects who responded to our initial publicity met criteria for DSPS. Of these, 123 (69%) were women and 55 (31%) were men. Mean age of responders (\pm S.D.) was 34.4 ± 10.4 years; mean age when their sleep schedules became a problem was 15.0 ± 10.8 years.

Sleep schedule problems interfered with work functioning in 67% of patients; 57% reported impairment in interpersonal relations. On average subjects reported going to bed at 1:25 a.m. on weekdays and 2:26 a.m. on weekends but noted that sleep onset occurred somewhat later (2:15 and 3:06 a.m. respectively). Corresponding average wake-up times for week and week-end days were 9:21 and 10:51 a.m. Most patients (84%) described the quality of their sleep as sound. Polysomnograms indicated that the primary sleep disturbance in DSPS patients was increased wakefulness in the first non-REM sleep cycle.

Four subjects were excluded because of coexisting sleep disorders. The other 18 showed only delayed sleep phase with otherwise healthy sleep. Nine subjects have been treated with both conditions thus far. Findings from these patients indicate that the bright light-dark goggles treatment was significantly more effective in shifting circadian rhythms earlier than the control treatment as measured by the clinical report and the morning sleep latency. Six of seven patients reported that bright light was more effective than dim light. Data for the 11:00 a.m. sleep latency are available for 6 patients before and after the bright condition, and on 4 patients before and after the dim condition. Values before and after the bright condition were 12.3 ± 7.5 minutes and 18.3 ± 6.4 minutes respectively ($P < .02$, Wilcoxon sign rank). There was no significant difference between latencies before and after the dim control condition. Post-treatment morning sleep latencies following the bright condition were significantly higher than those in the dim condition with values of 18.3 ± 6.4 minutes versus 13.0 ± 3.7 minutes ($P < .02$, Mann Whitney U Test). While morning alertness ratings and sleep-wake times also showed a shift in the direction of phase advance following the active treatment, they were not significantly superior to the effect of the control. Five of Six patients rated themselves as slightly more alert in mornings during the bright treatment as compared to the dim. Sleep logs appear to support the phase advance following bright treatment as compared to dim but the results were not significant. 24-hour temperature recordings and sleep studies require further analysis.

Significance to Biomedical Research:

Our data suggest that manipulation of the light-dark cycle can be used to therapeutic advantage in patients with DSPS who are motivated to change their sleep-wake cycle. In addition to helping individuals with chronic abnormalities in circadian rhythms, this information may be helpful in the treatment of the more common temporary shifts in circadian rhythms found in shift-workers and in jet-lag, both of which can produce dysphoria and functional impairment.

Proposed Course:

We plan to run a total of 20 DSPS patients through the crossover study to determine whether judicious manipulation of the light-dark cycle can indeed induce a statistically and clinically significant effect on the entrainment of their daily rhythms. In those people who are only partially helped we plan to use the short-acting benzodiazepine, triazolam, to improve compliance and enhance the phase-shifting effects of the treatment.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 MH 02205-03 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antidepressant Effects of Light in Seasonal Affective Disorder and Normal Controls

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N. Rosenthal	Chief, Unit on Outpatient Studies	CPB/NIMH
Others:	R. Skwerer	Medical Staff Fellow	CPB/NIMH
	K. Kelly	Medical Staff Fellow	CPB/NIMH
	S. Kasper	Visiting Associate	CPB/NIMH
	P. Schulz	Social Worker	CPB/NIMH
	S. Rogers	Registered Nurse	CC/NURS
	A. Yancey	Guest Worker	CPB/NIMH

COOPERATING UNITS (if any)

Pamela Madden, Guest Worker	Stephen B. Leighton, Sc.D
Michael Genhart, Guest Worker	Manuel Datiles, M.D., NEI

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

2.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously shown that exposure of the eyes to bright light, but not ordinary room light can reverse the winter depressive symptoms in patients with seasonal affective disorder (SAD). This light treatment may be effective during the morning, the evening or even during ordinary daylight hours. Normal subjects with no history of winter depression do not seem to benefit from conventional bright light treatment. However, those "normal" individuals who report some winter difficulties, albeit of a mild and subsyndromal nature, appear to benefit from phototherapy.

This past year we have evaluated the potential benefit of phototherapy for a representative sample of the general population as well as for a sample of healthy elderly individuals. In a study of 20 individuals chosen from the general population, we have once again shown that bright light treatment is not of benefit for the population at large. Similarly, in a study of 20 elderly subjects, we have shown that the elderly, as a group, do not benefit from this treatment. We have developed a new, more convenient, portable light source and have shown this to be effective in 6 patients with SAD. Thus, while confirming our original findings of the benefits of phototherapy in those carefully selected for a history of winter difficulties, and advancing the methodology for light administration, we have also confirmed our previous impressions that bright light is not a panacea to be advocated for the general population.

Project Description and Methods:

1. The Effects of Phototherapy in a Representative Sample of the General Population

This study was planned as a follow-up on the studies of the past two years, in which we showed that bright light therapy does not appear to be effective for those normal individuals without any history of winter difficulties. However, a segment of the normal population who does report having some problems with the winter, which are qualitatively similar to those reported by patients with SAD but far milder, do appear to benefit from such bright light therapy.

In the most recent study we recruited individuals from a sample of 416 people derived from an epidemiological study recently performed in Montgomery County (see Annual Report # Z01-MH 02422) according to the following guidelines: (1) We divided the total survey population into 20 categories according to the magnitude of the global seasonality score obtained from the Seasonal Pattern Assessment Questionnaire (SPAQ); (2) We aimed to interview 2 individuals from every category in order to obtain a homogeneous distribution according to the magnitude of the seasonality score; (3) Individuals reporting summer difficulties were excluded because the response of summer seasonal problems to phototherapy is unknown; (4) Individuals had to be in the age range between 30 and 60 years in order to obtain a homogeneous sample. One individual from each category was assigned to one of two light treatment conditions: bright (2500) and dim (300 lux) full-spectrum light treatment.

We compared the effects of 2 hours of bright light to 2 hours of dim light in the morning (between 6:00 and 9:00 a.m.) in 20 subjects in each group. Subjects were observed for one week before treatment, over one week of light therapy, and for one week after treatment. Subjects underwent light treatment in January and February 1988. Scales used for measurement of treatment effect included the 21-item Hamilton Depression Rating Scale, visual analog-type self-ratings, and the self-administered Profile of Mood States. Observer ratings were administered by raters blind to the treatment conditions. Results were analyzed by ANOVA with repeated measures and Pearson's correlations were performed between the change in mood score across treatment conditions and the retrospective seasonality score, in order to determine whether this score is predictive of response to light therapy.

2. Effects of bright light treatment in the elderly:

There is evidence that depression is one of the most commonly diagnosed psychiatric conditions in the elderly. Although depression is being diagnosed with more frequency and accuracy in this group, the degree of seasonal change in mood and behavior in the elderly remains unknown. As a result of many factors, such as failing vision and decreased exposure to natural light, the elderly would appear to be a population at high risk for decreased energy and depression in the winter months. In this study we evaluated the incidence of SAD in two residential housing complexes for senior citizens in the suburbs of Washington, D.C.

Volunteers for initial screening were recruited from these residences and were given the following screening tests: the Pfeiffer mini-mental status examination, the geriatric depression scale, the Seasonal Pattern Assessment Questionnaire (SPAQ), and the general health questionnaire with particular emphasis on ophthalmological history. Exclusion criteria for the light treatment study were: (1) Cognitive difficulties, as measured by the mini-mental status exam; (2) Major health difficulties, which would

preclude co-operation in the study; (3) Major visual problems, including a history of surgery for cataracts, poor vision, or active ocular pathology; (4) Unwillingness or inability for other reasons to co-operate with the treatment regimen.

Twenty senior citizens, chosen at random from the eligible pool of volunteers were given an extensive eye examination, involving visual acuity with refraction, slit lamp examination, keratometry, specular microscopy, dilated funduscopy, contrast sensitivity, glare measurement and Scheimpflug photography of the anterior segment of the eye. In addition, all participants had a complete physical examination with EKG and routine laboratory screening. These individuals were entered into a crossover study involving two treatment conditions, bright (2500 lux) and dim (300 lux) of full-spectrum light, administered for 5 hours per day (2.5 hours in the morning and 2.5 hours in the evening) for one week each. A week of withdrawal was allowed between the two treatment conditions. All treatments occurred in the winter of 1987-1988. Mood and behavior were assessed on a weekly basis by means of both observer ratings (21-Item Hamilton Depression Rating Scale) and self-ratings (Multiple Affect Adjective Checklist; MAACL). The data were analyzed by ANOVA with repeated measures.

3. Development and Testing of a Portable Light Source:

A major problem in the application of light therapy in the past has been the inconvenience and lack of portability of the light fixtures used to date. In order to address this problem, we constructed a portable light source from a welder's helmet, from the front of which a four-watt fluorescent tube is suspended. The upper portion and front of the fluorescent tube are covered by a reflective surface to increase the amount of light incident on the eyes. The subject is able to adjust the distance of the fluorescent light from the eye, and the resulting range of light intensities to which the eyes are exposed is between 2500 and 5000 lux. The current model requires being plugged into an electrical light source, but a long cord allows for considerable mobility, and the fact that it is attached to the subject means that movements of the head and body are not accompanied by corresponding changes in the amount of light to which the eyes are exposed.

Six patients with seasonal affective disorder (SAD) were given light treatment by means of this new portable device, for two hours each morning for one week, a treatment regimen that has previously been reported to be effective for this condition. Mood changes were rated by 21-item Hamilton Depression Ratings (with the 7-item supplement), administered by raters blind to the treatment condition.

Findings to date:

1. The Effects of Phototherapy in a Representative Sample of the General Population:

There was no beneficial effect apparent in the subjects in either bright or dim treatment conditions. Nor was there any correlation between effect of bright treatment and retrospectively evaluated seasonality score. However, there was a suggestion that those with highest seasonality scores did best on light treatment.

2. Effects of bright light treatment in the elderly:

A total of 140 screening interviews were conducted. The mean age of the population was 79 years. Ninety percent were white women; 66% were widowed; 79% had at least a high school education; 21% of those interviewed were mildly to moderately depressed, as measured by the geriatric depression scale, and 94% were within normal range on the Pfeiffer mini-mental status exam. The mean global seasonality score (\pm S.D.) of the SPAQ was 2.0 (\pm 2.5), a surprisingly low figure. Fifty-five percent of the interviewed subjects had never had any form of eye surgery.

Analysis of the Hamilton Rating score data revealed a significant interaction between bright and dim light treatments ($F=11.9$, $df=1, 15$, $P<.004$). Surprisingly, subjects did worse after bright than dim light. Mean (\pm S.D.) Hamilton Rating scores before and after the bright light condition were 2.6 ± 3.1 and 4.4 ± 4.1 respectively.

Corresponding scores for the dim light condition were 4.7 ± 3.1 and 3.7 ± 3.6 . Post-hoc comparison of Hamilton Rating scores before and after dim light treatment showed no difference. However, values were significantly higher after bright light treatment ($t=3.3$; $df=16$, $P<.005$).

3. Development and Testing of a Portable Light Source:

Six SAD patients with moderately severe depression (Mean Hamilton Depression Rating Score \pm S.D. = 18 ± 7) responded well to one week of treatment with the helmet, which lowered their rating scores to 2 ± 1 . Partial relapse was seen after a week of withdrawal from treatment to rating scores of 10 ± 5 . Patients found the helmet comfortable and more convenient than the traditional light box. No significant side-effects were reported.

Significance to Biomedical Research and to the Program of the Institute:

The findings that bright light is not helpful in treating a representative sample of the general population nor the elderly as a group corroborates our earlier impressions that such treatment is not universally helpful. These findings should make clinicians and public health workers cautious about extrapolating results of beneficial effects of bright light on selected clinical subgroups, to the population as a whole.

The portable light administering device promises to be an extremely valuable technological advance over the heavier and bulkier light fixtures currently in use.

Proposed Course:

The above formal studies of light treatment in non-depressed populations conclude our series in these particular individuals. Beyond the winter-SAD patients and subsyndromal SAD patients previously described to benefit from bright light therapy, who else may stand to benefit from this treatment? Recent data from Dr. Daniel F. Kripke's group in San Diego suggest that non-seasonally depressed individuals respond to light treatment. It would be of value to attempt to replicate their findings. Not only does this have potential clinical value, but it promises to enhance our understanding of how light therapy works, and the fundamental differences between seasonal and non-seasonal depressives.

Given the early promising results with the portable light fixture, it would certainly seem worthwhile to continue our work in this direction. Work is required on the fixture itself, which at present requires being plugged into an electrical outlet. A battery-operated portable model is in the process of being developed. We plan to study further the clinical value of these devices will be studied in the coming year.

Publications:

Hudson JJ, Pope HG, Jr., Wurtman JJ, Yurgelun-Todd D, Mark S, Rosenthal NE. Bulimia in obese individuals: Relationship to normal-weight bulimia. *Journal of Nervous and Mental Disease*. 1988;176(3):144-152.

Wehr TA, Rosenthal NE, Sack DA. Environmental and behavioral influences on affective illness. *Acta Psychiatrica Scandinavica*. 1988;77(341):44-52.

Kasper S, Wehr, TA, Rosenthal NE. Saisonal abhängige Depressionsformen (SAD).I. Grundlagen und klinische Beschreibung des Syndroms. *Nervenarzt* 1988;59:191-199.

Kasper S, Wehr, TA, Rosenthal NE. Saisonal abhängige Depressionsformen (SAD).II. Beeinflussung durch Phototherapie und biologische Ergebnisse. *Nervenarzt* 1988;59:200-214.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02206-04 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Seasonal Affective Disorder (SAD) and Light Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI.	N. Rosenthal	Chief, Unit on Outpatient Studies	CPB/NIMH
Others:	R. Skwerer	Medical Staff Fellow	CPB/NIMH
	F. Jacobsen	Medical Staff Fellow	CPB/NIMH
	K. Kelly	Medical Staff Fellow	CPB/NIMH
	T. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH

COOPERATING UNITS (if any)

Siegfried Kasper, Visiting Associate
Eric Souetre, Visiting Fellow

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although it has been established that bright light is an effective treatment for SAD, the mechanism of its action remains unknown, as do the fundamental biological abnormalities responsible for the syndrome. In previous years we have demonstrated biological abnormalities in patients with SAD and effects of light on a wide variety of biological systems. This past year we have extended these studies and, in particular, have conducted studies to examine two specific hypotheses involving the circadian system, and one involving the brain serotonergic systems. Circadian theories of SAD and phototherapy hold that there is an abnormality of either timing or amplitude of circadian rhythms, which is restored to normality by appropriately timed exposure to bright environmental light. We have shown abnormally low nocturnal secretion of melatonin, prolactin, TSH and growth hormone in SAD patients, which support the low amplitude hypothesis, as does our finding that the amplitude of the circadian rhythm of core body temperature is enhanced by bright light treatment. On the other hand, we have shown no abnormality in timing of circadian rhythms of these hormones, and no significant shift of these hormones following effective light therapy. Oral administration of the serotonin-2 antagonist, metergoline was given to eight SAD patients who were receiving phototherapy. No change of symptoms was seen with this drug, as compared to placebo, a finding that failed to advance further the serotonergic hypothesis of SAD and light therapy.

Project Description and Methods:1. Psychobiology of SAD and the Biological Effects of Light Therapy:

This study extends our work from the two previous winters. We have compared patients with SAD to normal controls on a number of biological measurements of interest. Since light is capable of producing rapid and marked antidepressant effects in SAD, we have compared patients in an untreated state and after treatment with bright light. Measures of interest studied this past year include: 24-hour profiles of plasma melatonin, prolactin, cortisol, TSH and growth hormone, and core body temperature.

SAD patients were followed longitudinally from the summer and fall, when they were euthymic, into the winter. As they became depressed, they were assigned to one of two conditions on a random basis: off-lights first or on-lights first, to avoid an ordering effect. In the former condition patients had been without light treatment for at least 10 days prior to being admitted for biological studies; in the latter condition bright light treatment had been administered for 2.5 hours in the morning (between 6.00 and 9.00 a.m.) and 2.5 hours in the evening (between 6.00 and 9.00 p.m.) for at least 10 days prior to admission.

Most of the patients were drug-free but a few patients on medications were included provided they had been on the medications for an extended period of time and the dosage could be held constant throughout the study. After the initial set of studies, patients were crossed over to the alternate condition. Age- and sex-matched controls were recruited and studied in a similar manner in the off-light condition. The results of hormone measurements and core body temperature were analyzed by ANOVA's with repeated measures.

2. The Administration of Metergoline to SAD Patients receiving light therapy:

A double-blind metergoline-crossover study was conducted in 8 patients with SAD after they had been treated successfully with two hours of bright (2500 lux) full-spectrum light in the morning for at least a week. The two conditions were randomly ordered and were administered during contiguous weeks. Metergoline (5 mg) and placebo were each administered one hour prior to bedtime for one week. Patient responses to both of these interventions were measured by means of the 21-item Hamilton Depression Rating Score, with the 7-item supplement to measure atypical depressive symptoms. Patients rated themselves by means of modified visual analog scales, NIMH self-rating scales, and side-effect checklists. Responses to drug and placebo, as well as side-effects, were analyzed by ANOVA with repeated measures.

Findings to Date:1. Psychobiology of SAD and the Biological Effects of Light Therapy:

In general, hormone data were available for 15 SAD patients on and off light treatment and for 11 normal controls, except in the case of TSH where data were available from only 7 patients and 7 controls. Overnight melatonin secretion was low in patients, who showed a mean overnight secretion (\pm S.D.) of 45.5 ± 4.3 pg/ml, as compared with normals, whose mean overnight secretion (\pm S.D.) was 71.1 ± 4.3 pg/ml ($p \leq 0.001$). Corresponding mean overnight secretions for prolactin, growth hormone and TSH in patients and normal controls respectively were 8.05 ± 0.33 and $11.7 \pm .76$ ng/ml ($p \leq 0.001$); 1.95 ± 0.21 and 3.34 ± 0.47 ng/ml ($p \leq 0.003$); and 1.90 ± 2.12 and 2.67 ± 1.57 μ U/l ($p \leq 0.006$). As we have previously reported, there was no

abnormality in the amount of cortisol secreted in SAD patients. There was no difference in circadian phase between patients and normals on any of the above hormones.

Phototherapy was highly effective in SAD patients, reducing Hamilton Rating Scores for Depression from a mean (\pm S.D.) of 22.3 ± 6.6 to scores of 6.8 ± 6.2 . This improvement in mood was not accompanied by any significant change in level or phase of any of the above-mentioned hormones.

Core body temperature measurements in 11 SAD patients and 11 matched normal controls were virtually identical. However, light therapy was accompanied by a significant lowering of core body temperature, which was not, however, accompanied by any change in circadian phase.

2. The Administration of Metergoline to SAD Patients receiving light therapy:

Patients did not show any differential response, good or bad, to metergoline as compared with placebo.

Significance to Biomedical Research and to the Program of the Institute.

The large number of abnormalities in circadian hormonal profiles confirm the clinical observation that major physiological disruptions are present in SAD. It is of interest that some of these hormones, most notably melatonin, prolactin and TSH are of importance in mediating seasonal rhythms in animals. The low amplitude of these hormones would support the low amplitude circadian rhythm theory of SAD. However, the failure of effective light therapy to reverse these rhythms might argue against their causal importance in producing the symptoms of SAD. The increased amplitude of the temperature rhythm following light therapy also supports the low amplitude circadian hypothesis. The normal circadian phase of all these rhythms and the absence of significant phase shift following effective light therapy would argue against the importance of phase shift in the pathogenesis of SAD or in the mechanism of action of phototherapy. However, it is possible that artifacts may have obscured or "masked" a true shift in circadian rhythms.

The negative result in the metergoline study does not offer further support to the serotonergic explanations of SAD and phototherapy. However, in view of other promising leads implicating this system, it would seem appropriate to continue to pursue serotonergic mechanisms in the future.

Proposed Course:

The proposed course in this particular study depends to some degree on data analysis that is yet to take place. Several studies were performed, the data of which are not as yet available as of the time of writing this report. These include a more sophisticated evaluation of the hypothalamo-adrenal-pituitary axis, namely measurement of the ACTH and cortisol responses to CRH infusions; and evaluation of satiety and cholecystokinin secretion following challenge with a test meal. Results of sleep studies, performed in conjunction with the hormonal studies, are also pending.

The challenge in deciding on future studies will be to choose which of the many available psychobiological leads will be most promising to follow. The circadian profile of core body temperature would certainly be worth studying in the summer months, as would the response of patients and normals to m-CPP. It would be premature to decide on the choice of optimal winter studies on the basis of the incomplete available information on studies performed thus far.

Publications:

Jacobsen FM, Sack DA, Wehr, TA, Rogers S, Rosenthal NE. Neuroendocrine response to 5-hydroxytryptophan in seasonal affective disorder. *Archives of General Psychiatry* 1987;44:1086-1091.

James SP, Sack DA, Rosenthal NE, Mendelson WB, Wehr TA. The effect of melatonin on normal sleep. *Neuropsychopharmacology* 1987;1(1):41-45.

Rosenthal NE, Jacobsen FM, Sack DA, Arendt J, James SP, Parry BL, Wehr TA. Atenolol in seasonal affective disorder: A test of the melatonin hypothesis. *American Journal of Psychiatry* 1988;145:52-56.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02290-04 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Melatonin Analysis of Clinical Samples

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Tamarkin

Research Biologist

CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Project held in abeyance.

The project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02292-04 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Melatonin Effect on Hormone-Stimulated Cell Growth

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Tamarkin

Research Biologist

CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project held in abeyance

The project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02294-04 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antidepressant Pharmacology of the Rodent Circadian System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. W. Duncan

Research Psychologist

CPB/NIMH

Others:

B. Gao

Fogarty Fellow

CPB/NIMH

L. Tamarkin

Research Biologist

CPB/NIMH

T. A. Wehr

Chief, Clinical Psychobiology Branch

CPB/NIMH

COOPERATING UNITS (if any)

P. Sokolove, Professor of Biological Sciences
University of Maryland

LABORATORY

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

.75

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Biological rhythms are disturbed in the majority of patients with affective disorder. These abnormal rhythms include disruptions of circannual or seasonal rhythms, as observed in seasonal affective disorder, disruptions of circadian or daily rhythms as observed in hypsomnia or hypersomnia, and disruptions of ultradian rhythms such as the disturbed REM cycle that frequently accompanies primary depression. This project focuses on the effects of antidepressant drugs on the mammalian circadian system; we have utilized the Syrian hamster as an animal model. We hypothesize that the mechanism of the antidepressant response to chronic drug therapy includes effects on the mammalian circadian system. Our results support this hypothesis.

Previously we reported that clorgyline, a monoamine oxidase inhibitor with antidepressant properties, altered the endogenous expression of the circadian system by a) delaying a large portion of motor activity to the second half of the activity phase, b) reducing the duration of the rest phase by about 30% and c) decreasing the frequency of the daily biological clock. We also reported that this drug altered the responsiveness of the daily clock to brief environmental light signals. In these past studies, we utilized wheel-running as an index of circadian pacemaker expression. During the past year we have extended our studies to include clorgyline's effects on EEG sleep and telemetered body temperature.

During the past year we have extensively explored the effect of clorgyline on the responsiveness of the circadian system to environmental light and dark. These studies indicate that during chronic clorgyline treatment a) the activity-rest cycle becomes progressively more disorganized as the intensity of continuous light is increased b) the capacity of the circadian system to respond normally to dark pulses is altered.

Project Description:

The activity-rest cycle is disturbed in the majority of patients experiencing depression and mania. The cause of this disturbance is not known but may include dysfunction of circadian oscillators within the mammalian system, and this dysfunction may contribute to depression. The antidepressant mechanism of drugs may include their ability to correct or otherwise alter this pathological substrate of the circadian system.

Many patients receiving drug treatment suffer further disruption of the activity-rest cycle during the course of antidepressant drug therapy. These side-effects may result from direct or indirect pharmacological actions on the circadian pacemaker that ultimately controls the expression of the sleep-wake cycle. The state of the circadian pacemaker may also be modified by the interaction of the pacemaker with the environment.

Our specific goals are to understand a) how drug treatment alters the intrinsic state of the mammalian circadian system and b) how drug treatment alters the response of the mammalian circadian system to the environmental conditions such as ambient lighting and temperature levels.

Methods

1) Experimental equipment

A description of the facility used to monitor the rodent circadian system can be found in project report Z01 MH 02294-01 CP. In addition we have been conducting EEG sleep studies with a Grass Model 78 Polygraph. Body temperature and motor activity are recorded using the Mini-Mitter telemetry system.

2) Antidepressant Drug Effects on EEG Sleep in Syrian Hamsters

In this study, the EEG and EMG of hamsters are recorded for selected twenty-four hour intervals for over one month. During this time they are treated with clorgyline (2 mg per kg per day) using subcutaneously implanted Alzet mini-osmotic pumps. Surgery is performed using either halothane or pentobarbital anesthesia. The twenty-four hour records are visually read and analyzed using a laboratory computer. Some of these animals are also implanted with an intraperitoneal transmitter in order to monitor body temperature.

3) Antidepressant Drug Effects on Telemetered Body Temperature in Syrian Hamsters

Hamsters are implanted with intraperitoneal transmitters in order to simultaneously monitor body temperature and motor activity. Hamsters are also treated with either saline or clorgyline (2mg per kg per day) using Alzet mini-osmotic pumps. Data are collected online and analyzed using a laboratory computer. To date these preliminary studies have been conducted in LD 14:10.

4) Interaction of Drug Effects and Constant Lighting on Wheel-Running in Syrian Hamsters

Hamsters are treated with clorgyline as described above. Following a pretreatment period with clorgyline, hamsters are isolated in individual cages with access to running wheels. Following a baseline interval in LD 14:10, hamsters are maintained in continuous lighting (LL) for several weeks. Three different light intensities have been studied: 1-2, 5-6, and 20-40 $\mu\text{W cm}^{-2}$. During the course of this experiment a dark-pulse is administered to the hamsters that have otherwise been housed in LL.

Findings to date:

1) Antidepressant Drug Effects on EEG Sleep in Syrian Hamsters

Clorgyline alters the EEG sleep pattern of hamsters studied in a light-dark cycle. As expected, but not previously reported in Syrian hamsters, this MAOI significantly decreases REM sleep. A second significant observation is that clorgyline increased EEG documented wakefulness during the second-half of the hamsters' activity phase. Last year we reported that clorgyline increases wheel-running activity during the second-half of the activity phase. These observations suggest that clorgyline's effects on EEG wakefulness and motor activity are phase dependent. We interpret these findings as indirect evidence that clorgyline alters the "M-oscillator" which controls the expression of both morning motor activity and morning EEG arousal.

2) Antidepressant Drug Effects on Telemetered Body Temperature in Syrian Hamsters

Preliminary data indicate that chronic clorgyline treatment produces hypothermia in Syrian hamsters. This effect appears to be relatively uniform throughout the circadian cycle. The magnitude of the drop is about .5 degrees centigrade.

3) Interaction of Drug Effects and Constant Lighting on Wheel-Running in Syrian Hamsters

We observed that clorgyline alters the response of Syrian hamsters wheel-running in constant ambient lighting. The qualitative aspects of this interaction were dependent on the intensity of ambient lighting. During constant, low intensity lighting, clorgyline increased the period of the daily biological clock compared to control animals. During constant, high intensity lighting, clorgyline also initially increased the period of the clock. However, the activity-rest cycle eventually became dissassociated and/or arrhythmic in all clorgyline-treated animals studied in high intensity light. Control animals in high intensity light exhibited only an increase in the period of the clock.

In a related experiment, hamsters studied in dim light were exposed to a six hour pulse of darkness during the middle of the rest phase. In contrast to saline-treated hamsters, clorgyline-treated hamsters exhibited an attenuated response. These data can be interpreted to indicate either a) clorgyline delayed the phase of the circadian pacemaker in LL, consistent with its effect on the pacemaker when studied in constant darkness or b) clorgyline altered the responsiveness of the circadian pacemaker to darkness.

Significance to Biomedical Research:

This project is significant to biomedical research for two reasons. First, we have clearly identified the circadian pacemaker as a target for the MAOI clorgyline, a drug that has antidepressant properties in humans. Further we have begun to identify the times during the circadian cycle when the drug seems to exhibit maximal activating effects. Second, high levels of ambient lighting in combination with MAOIs may result in disruptions of the human sleep-wake cycle, since sleep disruption is known to trigger mania, these effects of clorgyline on the sleep-wake cycle may be partly responsible for clorgyline's capacity to induce mania.

Proposed Course:

There are three directions to be taken during the coming year. The first is to localize the target for clorgyline's action on the mammalian circadian system. We will combine our observed interaction between chronic clorgyline treatment and bright light with lesion studies (particularly the intergeniculate leaflet of the lateral geniculate nucleus and the 5HT projection to the SCN) in order to localize clorgyline's capacity to alter visual responsiveness. Second we will continue to conduct circadian studies using EEG documented sleep, core body and brain temperature, and motor activity in order to determine the hierarchy of drug input to the mammalian circadian system. Finally we will extend our studies to include the interaction of ambient temperature and light on the mammalian circadian system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02303-02 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Sleep in Psychiatric Illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. Mendelson

Chief, Section on Sleep Studies

CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Section on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.3

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

In a previous study patients with bipolar depression were compared with age and sex matched controls; there were differences in sleep efficiency and total sleep, but no differences in REM latency or power spectra. We compared the power spectra of insomniacs and controls, and found no difference. Our previous studies of depressed bipolar patients compared with normal controls showed differences in sleep efficiency and total sleep but no difference in REM latency and power spectra. We examined the power spectra of the sleep EEG in insomniacs and controls, and found no difference.

This project was terminated in July, 1987 when the principal investigator in the Section on Sleep Studies left the NIH.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02324-03 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuroendocrine Modulation of Cellular Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Tamarkin

Research Biologist

CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Project held in abeyance.

The project has been terminated.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02325-03 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Light and Lymphocyte Activity: Basic and Clinical Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Tamarkin

Research Biologist

CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project held in abeyance and data from last year submitted for publication or in preparation.

The project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02326-03 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Modulation of the Cellular Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Tamarkin

Research Biologist

CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project held in abeyance.

The project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02327-03 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Direct Effect of Lymphokines on Cultured Human Breast Cancer Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Tamarkin Research Biologist CPB/NIMH

Others: G. Paciotti Biologist

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Interleukin 1 has been shown by us to have a direct effect on hormone dependent breast cancer cells; the mechanism by which this occurs appears to be through an IL-1 receptor on these cells. During the last year the focus of this project has been on studying the effect of IL-1 on cell growth. This has been accomplished by using flow cytometric analysis of the cell cycle. These data indicate that IL-1 specifically arrests cells in the G0/G1 phase of the cell cycle. We have used this phenomenon to demonstrate increased cytotoxicity by the chemotherapeutic agent, 5-fluorodeoxyuridine. These data indicate that IL-1 has direct effects on cells outside the immune system and that it may play a role in synchronizing cells in the G0/G1 stage of the cell cycle.

The project has been terminated.

Project Description:

In last year's report the longterm effect of IL-1 on in vitro cell growth was described. This year we report the identification of an IL-1 receptor on hormone dependent breast cancer cells that is of the same molecular weight as the receptor identified on T-cells; a binding protein could not be identified on the hormone independent cell lines. Additionally, we have characterized the effect of IL-1 on cell growth by examining the effect of IL-1 on the progression of cells through the cell cycle. Using propidium iodide we have determined that IL-1 arrests the cells in the early stage of the cell cycle and that this is accompanied by the induction of a 27,000 molecular weight protein.

Publication:

Paciotti GF, and L Tamarkin. Interleukin 1 directly regulates hormone-dependent human breast cancer cell proliferation in vitro. *Molecular Endocrinol* 1988;2:459-464.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02328-03 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Direct Effects of IL-2 on Cultured Anterior Pituitaries

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Tamarkin

Research Biologist

CPB/NIMH

Others: M. Collins

Biologist

CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The role of the pituitary hormone, ACTH, on macrophage has been the focus of this project. The results indicate that ACTH can stimulate macrophage cell lines to grow in vitro and that ACTH in conjunction with lipopolysacchride (LPS) can cause the secretion of tumor necrosis factor. The mRNA has been isolated from these stimulated cells to determine if the stimulation of TNF secretion occurs at the genetic level.

The project has been terminated.

Project Description:

The project tests the hypothesis that ACTH directly stimulates macrophage. The approach has been to investigate this hypothesis in vitro. Specifically, cell growth was assessed and the secretion and genetic expression of the message for two macrophage proteins, interleukin 1 and tumor necrosis factor

Methods:

A dose dependent effect of ACTH on 3H-thymidine uptake by four macrophage cell lines, IC-21, J774, HL-60, and U937. Primary macrophage do not grow in vitro and were not tested for in vitro growth.

Functional assays developed to assess ACTH effects on TNF and IL-1:

TNF bioassay performed on WEHI clone 13 cell line, sensitivity of .25 pg/ml. IL-1 bioassay using primary murine thymocytes, sensitivity of 50 pg/ml.

Findings to date:

ACTH causes the above cell lines to grow in a dose dependent manner. ACTH in conjunction with LPS causes the secretion of TNF, no effect on IL-1 was observed.

Significance:

Endocrine hormone directly affecting an immune function, suggesting cross-talk between the neuroendocrine and immune systems.

Proposed course:

Determine if the mRNA for TNF is stimulated by ACTH and LPS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02402-02 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Causes and Treatment of Summer Depression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH
Others:	N. E. Rosenthal	Chief, Unit on Outpatient Studies	CPB/NIMH
	P. Schulz	Social Worker	CPB/NIMH
	S. Kasper	Visiting Associate	CPB/NIMH
	K. Kelly	Medical Staff Fellow	CPB/NIMH
	J.R. Joseph-Vanderpool	Medical Staff Fellow	CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There appear to be two, opposite seasonal influences on the occurrence of depression: patients are more likely to become depressed in the late spring and early summer and in the late fall and early winter than at other times of year. These seasonal pattern are of particular interest because they suggest that environmental factors may be capable of causing and terminating affective episodes.

Considerable evidence now suggests that recurrent winter depressions are caused by light deficiency and that they can be treated with augmentation of light exposure. In this project we are investigating depressive symptoms and their responses to environmental manipulations in patients with recurrent summer depressions. Patients with summer depression are more likely than patients with winter depression to have endogenous symptoms, such as decreased appetite, decreased weight, decreased sleep, and psychomotor agitation. In contrast, patients with winter depression are more likely to have so-called atypical symptoms, such as increased appetite, carbohydrate craving, increased weight, increased sleep, and psychomotor retardation. We tested the hypothesis that seasonal changes in light or temperature might cause summer depression, in two experiments carried out at two different times of year. When patients were depressed in the summer, the possible therapeutic effects of darkness and cold were compared in a balanced randomization crossover study. The results of the study were encouraging, in that patients improved significantly after both types of treatment. However, the results did not enable us to distinguish between the respective roles of light and temperature. When patients were well in the winter and early spring, we conducted a complementary study in which we found that heat and humidity, but not light, appeared to induce symptoms of summer depression. We also found that thyroid hormones decreased when patients became depressed in the summer.

Project Description:

Affective illness is inherently recurrent, and there is considerable evidence that patterns of recurrence in affective illness are strongly influenced by seasonal factors. Specifically, episodes of depression are more likely to begin in the spring and in the fall than at other times of year. Sometimes episodes recur on an annual basis producing regular patterns of summer (spring onset) or winter (fall onset) depressions, conditions we have termed Seasonal Affective Disorders (SAD). Seasonal influences are important because they suggest that depression might be caused by changes in the physical environment and that depression might be treated by manipulations of the physical environment. For a number of years we have investigated depressions that regularly recur in the winter, and there is now substantial evidence that these depressions may be caused by deficiency in light and that they can be treated by augmentation of light exposure.

About two years ago we identified a population of patients who regularly became depressed in the summer. We have investigated clinical features of summer depression, and we have tried to identify possible environmental causes of summer depression by assessing patients' responses to experimental manipulations of environmental temperature and light during their depressed state and during their well state.

Methods:

Patients were recruited by referral by clinicians familiar with our studies of seasonal depression or by descriptions in the media of our research program. Patients' diagnoses were based on the Revised Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-III-R) using data obtained with the Structured Clinical Interview (SCID). Their clinical state was assessed with the Hamilton Depression Rating Scale (HDRS), the Weekly Mood Inventory (WMI), and visual analogue rating scales (VAS). For comparison, a group of patients with recurrent winter depressions was evaluated with the same instruments.

Thyroid axis hormones (known to be influenced by temperature changes) were measured.

Clinical and biological changes in the patients were monitored prospectively through the four seasons of the year, beginning in spring of 1987.

In addition, patients participated in two experiments designed to evaluate the possible role of seasonal changes in environmental temperature and light as causes of their summer depressions.

Experiment 1: In the summer, when patients became depressed, they were exposed to two treatment conditions: 1) isolation from bright light (with special neutral density glasses) and exposure to darkness, and 2) isolation from heat (with airconditioning) and exposure to 40° F. The treatments were carried out during two different five day periods, with time between the periods to allow for relapse if patients improved. Dark and cold exposures lasted twenty minutes and were repeated four times a day. For an additional twenty minutes four times a day patients were exposed to outdoor heat in the dark condition and outdoor light in the cold condition. Clinical state before and after treatments and after withdrawal of treatments was assessed by blind raters using the HRSD and by the patients using the VAS. Patients' expectations of the two treatments were assessed before the treatments began.

Experiment 2: In the late winter and early spring, when patients were well, they were exposed to two experimental conditions: 1) exposure to bright light and 2) exposure to heat and humidity. The experiments were carried out during two different two day periods, with time between the periods to allow the patients to return to their baseline state. Clinical state before and after experimental conditions and after withdrawal of experimental conditions was assessed by blind raters using the HRSD and by the patients themselves using the VAS. Patients' expectations of the two experimental conditions were assessed before each condition began, and their evaluations of the two experimental conditions were assessed at the end of each

condition.

Positron Emission Tomography (PET) scans were obtained during summer depressions and after cold treatment.

Findings to Date:

1. Contrasting clinical features of summer depression and winter depression. Twenty patients with summer depression were compared with twenty patients with winter depression. All patients met the following criteria: a) Their DSM-III-R diagnoses were assessed with the structured SCID interview; b) They were prospectively observed to become depressed during the season when they usually became depressed; c) When they became depressed, their depressive symptoms were assessed with the HRSD; d) They completed the SSQ and SPAQ self-assessment forms detailing the history and seasonal patterns of their illness. Results: Patients with winter depression were more likely to have so-called atypical features of depression, with increased appetite, carbohydrate craving, weight gain, increased sleep, and psychomotor retardation. Patients with summer depression were more likely to have endogenous features of depression, with decreased appetite, weight loss, decreased sleep, and psychomotor agitation. Twenty-five percent of patients with summer depression also had co-existing lifetime diagnoses of anxiety disorders (panic disorder, agoraphobia, social phobia), whereas none of the patients with winter depression had anxiety diagnoses. About half of the patients in each group had a bipolar form of affective illness (mostly bipolar II with hypomania) and half had a unipolar form.

2. Biological correlates of seasonal mood changes in summer depressives. Thyroid axis hormone levels (TSH, T3, T4, and Free T4) showed statistically significant seasonal variations in the summer depressives, with lowest values in the summer and highest values in the winter. Seasonal variations in thyroid axis hormones in normal subjects are being assessed in order to determine whether the seasonal variation in patients is physiological or pathological in degree. PET scans are not yet analyzed.

3. Patients' responses to experimental manipulations of environmental temperature and light.

Experiment 1. Treatment of summer depression by reducing exposure to environmental heat or light. Eight patients agreed to participate in the experimental cold versus dark treatment study. Patients significantly improved after both types of treatment with approximately 50% reductions in HRSD scores. There are several possible explanations for this result.

- a. Both temperature and light are capable of regulating mood
- b. The effects of temperature and light were confounded in the study
- c. Some other factor(s) that were not controlled in the study (e.g., hospitalization) were responsible for the improvements.

Experiment 2. Effects of environmental heat and light on remitted summer depressives in the winter and early spring. Eight patients agreed to participate in the experimental exposures to heat and humidity versus bright light. As assessed by the HRSD, the patients' clinical state worsened during the hot, humid condition but not during the bright light condition. Hamilton depression ratings during the hot, humid condition approached values that were seen during spontaneous relapses in the summer, and differences between the heat and light conditions were statistically significant. Patients' expectations were predictive of their responses.

Taken together, these results suggest that increased heat and humidity in the summer may trigger summer depressions, and that manipulations of temperature and humidity might be used to treat or prevent this condition. These possibilities require further investigation.

Significance to Biomedical Research:

Studies of seasonal depression provide a unique opportunity to explore the possible role of

physical environmental factors, such as light and temperature, as causes of and treatments for depression. This type of research has already led to the development of a new type of antidepressant treatment, phototherapy, for depression, and has stimulated a series of basic studies of biological effects of occularly mediated light as possible mechanisms. In the same way, proof of effects of temperature on clinical state could be expected to lead to additional new types of treatment for depression and mania, and to new knowledge about the biological effects of environmental temperature.

The association of endogenous symptoms with summer depression and atypical symptoms with winter depression raises the possibility that these two contrasting types of depression may be caused by exaggerated responses of physiological mechanisms that normally facilitate the organism's adaptations to conditions that prevail in the summer and winter, respectively.

Drugs that modify the state of neural systems which are sensitive to environmental temperature and light might be employed to treat depression and mania.

Proposed Course:

To further investigate the possible role of temperature and/or light as causes of, and treatments for, depression and mania, summer depressives will be treated experimentally with exposures to reduced environmental temperature and reduced light intensity while they are depressed in the summer. The design of the previous summer's treatment study will be improved by intensifying the contrasts between the two conditions.

The finding that thyroid hormone levels decline as patients become depressed in the summer will be followed up by more detailed investigations of the thyroid axis in these patients and in normal individuals through the course of the year.

Publications:

Wehr TA, Sack DA, Rosenthal NE. Seasonal affective disorder with summer depression and winter hypomania. *American Journal of Psychiatry* 1987;144:1602-3.

Wehr TA, Sack DA, Rosenthal NE. Environmental and Behavioral Influences on Affective Illness. *Acta Psychiatrica Scandinavica, Supplementum* 1988;77(341):44-52.

Wehr TA. Seasonal affective disorders: an historical overview, in Rosenthal NE, Blehar M (eds), *Seasonal Affective Disorders and Phototherapy*, New York, Guilford Press, in press.

Wehr TA, Giesen H, Schulz PM, Joseph-Vanderpool JR, Kasper S, Kelly K, Rosenthal NE. Summer depression: Description of the syndrome and comparison with winter depression. In Rosenthal NE, Blehar M, eds. *Seasonal Affective Disorders and Phototherapy*, New York, Guilford Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02403-02 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Action of Melatonin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Tamarkin

Research Biologist

CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)



(a) Human subjects



(b) Human tissues



(c) Neither



(a1) Minors



(a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Project held in abeyance.

The project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02405-02 CP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Chemical Antidepressant Effects on Body Mass and Body Composition in Hamsters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. Duncan Research Psychologist CPB/NIMH
Others: T. A Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

COOPERATING UNITS (if any)

T.J. Bartness, Senior Research Associate,
Worcester Foundation Experimental Biology

LAB/BRANCH
Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: .5	PROFESSIONAL: .25	OTHER: 0
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CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antidepressant drugs are often observed to produce changes in the body mass of patients receiving treatment. We have previously observed that chronic treatment with the monoamine oxidase inhibitor clorgyline alters the body mass of Syrian hamsters. Hamsters treated with this drug failed to increase body mass as did control hamsters. The change in body mass was primarily due to an effect on body lipid content. In our earlier studies we also observed that the failure to increase body mass was not due to a decrease in caloric input since clorgyline-treated hamsters exhibited greater food intake than saline-treated hamsters. Thus, clorgyline produced a condition of negative energy balance compared to control hamsters.

To more fully examine the interaction of environment, behavior and metabolism in antidepressant drug-treated hamsters, we have designed a chamber that will allow online, simultaneous measurement of oxygen consumption, motor activity, food intake and body temperature. Ambient temperature can be controlled between 5 and 40 degrees centigrade. Ambient lighting can be programmed to follow numerous regimens including seasonal fluctuations in daylength. The relationship between parameters which determine energy balance, i.e., energy lost as heat (either during motor activity or during thermoregulation), or energy gained during food intake, can be explored within a circadian perspective.

Project Description:

Antidepressant drug treatment is associated with changes in body mass and energy levels in depressed patients. We have observed changes in these parameters in drug-treated hamsters. Our objective is to describe within a circadian framework, those factors responsible for altering energy balance in drug-treated hamsters.

Methods:

Experimental equipment

1) General

This system is capable of monitoring the metabolic rate and behavior of four animals simultaneously. Each metabolic chamber measures approximately 44 x 44 x 31 cm (w x l x h) and is air tight except for a controlled air supply and return. The test animal may be housed in this chamber for several weeks during which motor activity, food consumption, oxygen consumption and body temperature is continuously collected and stored on a laboratory computer. This system permits collection of multiple parameters which vary in a circadian pattern.

Ambient temperature is regulated by a temperature controller which operates within a range of five to forty degrees centigrade. The humidity of the chamber is controlled by driers that operate as part of the air pump.

Lighting parameters are programmed using the microprocessor -based Chronotrol timer.

2) Motor activity

Motor activity is collected by counting the total crossings of a series of infrared beams and detectors which surround the perimeter of the metabolic chamber. The total crossings are counted per unit time and stored in the laboratory computer. The system may be modified slightly to record EEG sleep.

3) Food intake

Food is provided to the animal on a dish that rests on an automatic balance. Animals are prevented from removing food from the dish except for that which is immediately consumed. Food consumption is calculated as the difference between the food mass present on two consecutive sampling intervals. These data are automatically stored on a laboratory computer.

4) Body temperature

The core body temperature is measured by telemetry. A temperature dependent signal is transmitted to a receiver board located under the metabolic chamber, and stored on the computer.

5) Oxygen consumption

Oxygen consumed by the experimental animal is determined by online calculation of the difference between the oxygen concentration of air supplied versus the oxygen concentration of the air returned. Air flow is controlled by pumps which regulate the supply velocity and time constant of the oxygen response.

Findings to date:

Since this system has been in development and has only recently become operational, we have no findings to report.

Significance to Biomedical Research:

In humans, antidepressant drugs are often observed to produce the unwanted side-effect of increased weight gain. This response is not understood but may result from a shift in the thermoneutral set point or a change in diet. It is possible that the change in body mass is part of, or a clue to the mechanism of the antidepressant response itself. The Syrian hamster may be a suitable model to explore the metabolic effects of antidepressant drugs. It is anticipated that the multiple channel, rodent metabolic system will advance our understanding of the mechanism of the antidepressant drug response and assist in the development of new treatments that do not exhibit unwanted side-effects.

Proposed Course:

The mechanism of the antidepressant response to antidepressant drug treatment may include alterations in energy balance. Our preliminary data indicate clorgyline treated hamsters are in a negative energy balance. These data are consistent with the view that clorgyline shifts the set-point for temperature regulation further from the thermoneutral zone, therefore increasing energy demand. In non-pharmacological studies, total REM sleep decreases as the difference between the zone of thermoneutrality and ambient temperature increases. Our preliminary data indicates that clorgyline decreases REM sleep in Syrian hamsters. These data are consistent with the view that clorgyline alters the zone of thermoneutrality. We will evaluate the effect of clorgyline, as well as other monoamine oxidase inhibitors and tricyclic antidepressant compounds on a) the circadian pattern of oxygen consumption and b) the zone of thermoneutrality. Our hypotheses are that the metabolic effects of these drugs on behavior and physiology are circadian-phase specific, and that by manipulating ambient conditions such as temperature, the metabolic (antidepressant) effects may be amplified or diminished.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02422-01 CP
PERIOD COVERED October 1, 1987 - September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of the pattern of seasonal changes of mood and behavior in the general population.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	N. Rosenthal	Chief, Unit on Outpatient Studies CPB/NIMH
Other:	S. Kasper	Visiting Associate CPB/NIMH
COOPERATING UNITS (if any)		
Steven Targum, Sarasota Palms Hospital Howard Hoffman, Psychiatric Inst. of Washington		Leora Rosen, USUHS Michael Terman, Columbia Hospital
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	0.5	2.0
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>Three studies were performed to evaluate the prevalence of <u>seasonal changes</u> in the <u>general population</u>: a telephone survey in Montgomery County, a mail-out survey at four latitudes along the East Coast, and a survey in doctors' offices at three out of the four latitudes mentioned above. All studies used the Seasonal Pattern Assessment Questionnaire (SPAQ) previously developed by our group. The general population studies showed that seasonal changes are a problem for approximately one quarter of the population. At the further northern <u>latitudes</u> more people complain about winter, whereas in the most southern latitude (Sarasota, Florida) complaints about summer were commoner. We derived case finding criteria for SAD, subsyndromal SAD (S-SAD) and summer SAD from the SPAQ, and on the basis of these criteria, found the incidence of these three conditions to vary between 1.4-8.9%, 2.6-11% and 0.5-1.2%. There was a significant correlation between the prevalence of S-SAD and latitude, and S-SAD plus SAD, and latitude. The latter figure is of particular interest as it encompasses those individuals who are likely to benefit from enhanced environmental lighting. Extrapolating from the prevalence-latitude curve we estimate that this last combined group may number as many as 36 million people in the continental United States.</p> <p>This process has been completed and the project is terminated.</p>		

Project Description and Methods:

Previous studies of SAD and subsyndromal SAD have relied on recruitment of patients via the media. Although volunteers have been available in large numbers at centers across the northern United States and Europe, no systematic population studies of seasonal problems in mood and behavior have been published to date. We have attempted to examine this area in the past year.

We conducted three studies to examine seasonal variations of mood and behavior among the general population. All three studies employed a version of the Seasonal Pattern Assessment Questionnaire (SPAQ) (Rosenthal, Bradt and Wehr, 1984), but they differed in scope, target population, and method of administration of questionnaires. In one study, the general population of Montgomery County was sampled by random digit dialing and a telephone version of the SPAQ was administered to the target population. In the second study, SPAQs were mailed to samples of the general population derived from telephone directories in urban and suburban settings at four latitudes along the East Coast of the United States. In a third study, questionnaires were handed out to patients in doctors' offices at three of the four sites used for the mailout study.

1. The Montgomery County Study

In an effort to obtain information on seasonal variations in behavior in a suburban setting, we conducted a telephone survey of a representative sample of the general population of Montgomery County, Maryland. The major objectives of this survey were (1) to describe the pattern and degree of seasonal changes in mood and behavior in the general population, (2) to compare characteristics of individuals with winter- and summer-patterns of seasonal change, which might be related to the different symptoms of winter- or summer-SAD (see Annual Report #ZO1 MH 02402-02 CP), (3) to calculate prevalence rates for the occurrence of winter SAD and subsyndromal SAD, and summer SAD, and (4) to study the relationship between age and seasonality, and sex and seasonality.

Completed questionnaires were obtained from 416 subjects, which constituted a response rate of 92%. Clinical evaluations were performed on a representative subset of 40 subjects of varying degrees of seasonality, as measured by the Global Seasonality Score on the SPAQ. We attempted to develop SPAQ-based case finding criteria for the diagnosis of SAD and S-SAD and compared prevalence figures derived in this way for the general survey population with figures obtained in the same way for the clinically evaluated subset.

2. The Four-Latitude Mailout Study

Given the responsiveness of SAD, subsyndromal SAD, and summer SAD to the physical environment, their prevalence would be expected to differ at different latitudes. The purpose of this study was to evaluate the distribution and prevalence of seasonal changes, including their extreme manifestations, SAD, S-SAD and summer SAD, at four different latitudes. In the winter of 1987-1988 the Seasonal Pattern Assessment Questionnaire (SPAQ) was sent to 1000 people chosen randomly from telephone directories in Nashua, New Hampshire (42.5°N); Montgomery County, Maryland (39°N); and Sarasota, Florida (27°N); together with an introductory letter. An attempt was made to balance the sexes and only subjects older than 16 were included. Two follow-up letters were sent to increase response rate. The methodology was patterned on a mail-out survey in which 400 SPAQs had been sent out in New York City (40°N) the previous winter (Terman, 1987), the results of which are included in the present report. We estimated the prevalence of SAD, S-SAD and summer SAD at the different sites by using criteria developed in the telephone survey described above. We correlated prevalence of SAD, S-SAD and summer SAD with latitude at the four different sites.

3. The Three-Latitude Doctors' Office Study

The purpose of this study was to assess seasonal patterns of mood and behavior in patients visiting non-psychiatric doctors' offices at different geographical latitudes. Results of the Four-Latitude Mailout Study strongly supported the hypothesis that rates of SAD increased with increasing latitude north of the equator. However, with a growing body of literature suggesting that psychiatric illnesses are usually seen and treated by the family physician, at least in the early stages, it seemed warranted to explore the extent to which seasonal disorders appear in such doctors' offices.

Eleven physicians at three centers (Nashua, NH; Washington, D.C.; and Sarasota, FL) agreed to participate in the study. All were internists, general practitioners or family practitioners, including two from Nashua, one from D.C., and eight from Sarasota. Comparisons were made between 559 patients from this study and 1384 non-patients whose data was obtained from the Four-Latitude Mailout Study.

Findings to Date:

1. The Montgomery County Study

We found that 92% of the survey population noticed seasonal changes of mood and behavior to varying degrees. For 27% of the sample population seasonal changes were a problem and 5 to 10% of the general population - depending on the case finding definition - rated a degree of seasonal impairment equivalent to that of patients with seasonal affective disorder (SAD). The seasonal pattern of "feeling worst" exhibited a bimodal distribution with a greater winter and a substantially lower summer peak (ratio 4.5:1). The results of multivariate techniques revealed that younger women, between 21 and 40 who have a problem with the changing of the seasons and who feel worse on short days tended to exhibit the highest seasonality scores. We have proposed criteria for identifying SAD patients in the survey population and estimated point prevalence rates. According to this definition, we found a prevalence rate of 4.3% for SAD patients with a winter-pattern and 0.7% for SAD patients with a summer-pattern. In both groups there was a substantially higher percentage of women. A further 13.5% of the survey population were classified as having a subsyndromal winter SAD pattern. Prevalence rates based on the clinical evaluations of the 40-subject subsample suggest the the above estimates of SAD and S-SAD are probably conservative. Winter seasonals tended to dislike grey, cloudy days and cold weather. Summer seasonals tended to feel worse on long days, sunny days, and during hot weather. Also, while total sleep time was relatively stable for summer types, there was a higher fluctuation in the winter-pattern group, with longer sleep occurring in the winter months. Lastly, winter-types reported changes in food preferences with changes in the seasons, characterized by the preference of high-carbohydrate foods when feeling worst. A significant negative correlation between age and GSS was found for females. As women got older, they appeared to become less seasonal, indicating a possible hormonal mechanism in seasonality, whereas in males, seasonality appeared to remain basically constant throughout the lifespan.

2. The Four-Latitude Mailout Study

Survey response rates for New Hampshire, New York City, Montgomery County and Florida were 40.1%, 57.1%, 60.5% and 45.9% respectively. Sex ratios were approximately equal at all centers except for New Hampshire, which had a female:male ratio of 3:2. Mean scores (\pm S.D.) of the Global Seasonality Score (GSS) for NH, NY, MD and FL were 7.6 ± 4.6 , 7.7 ± 4.6 , 6.7 ± 4.3 and 5.4 ± 4.1 respectively. Scores for NY and NH differed from that for MD ($p < .05$), which in turn differed from that for Florida ($p < .05$). There was a tendency for more seasonal problems to occur in the northern latitudes, where subjects reported feeling worst predominantly in the winter. Subjects in Florida tended to report feeling worst in the summer. At all four centers there was a tendency for people to eat more, gain weight, sleep more and have less energy in the winter than in the summer, changes that are in the same direction as those seen in SAD. The

percentages of people in Sarasota, Montgomery Co., and Nashua who noted changes in the seasons to be a problem in their lives were 14%, 22% and 26%, respectively, with greater percentages reporting problems the further north the site ($X^2 = 21.3, p < .001, df = 3$).

The prevalence of SAD for the four centers, as determined by SPAQ-based criteria derived from the Montgomery County study described above, was 1.4% in FL, 6.3% in MD, 4.7% in NY, and 8.9% in NH. For S-SAD, these percentages were 2.6%, 10.4%, 12.4%, and 11%, respectively. There was a strong correlation between latitude and prevalence of S-SAD ($r = 0.97, p < .01$), and between latitude and a combination of prevalence of SAD and S-SAD ($r = 0.99, p < .01$). The correlation between latitude and prevalence of SAD fell just short of significance ($r = 0.87$). Sex ratios for the different case types at the different centers were generally balanced. Only in Maryland was the preponderance of females significant for SAD ($p < .0001$). Only in Florida was the preponderance of females significant for S-SAD ($p = 0.05$). The prevalences of summer-SAD in NH, NY, MD and FL were 0.5%, 3.1%, 1.0% and 1.2% respectively. There was no correlation between latitude and prevalence of summer-SAD as defined by our criteria.

A regression line based on the distribution of SAD and S-SAD across latitudes was used to estimate the prevalence of these conditions for different latitude bands, and an overall prevalence of these conditions for the U.S. was estimated. If these methods truly reflect population prevalence rates, and if the responses at the four sites are representative of those elsewhere in the United States, our data suggest that there are approximately 10.8 million cases of SAD and 25.3 million cases of S-SAD in this country. Since both of these groups have been shown to benefit from enhanced environmental lighting, we estimate the total number of people in the continental U.S. who may stand to benefit from this intervention to be approximately 36.1 million.

3. The Three-Latitude Doctors' Office Study

There were perfect negative correlations between the prevalence of summer SAD and latitude in both the doctors' office sample and the general population sample at the three locations: Nashua, Washington, D.C./Montgomery County, and Sarasota ($r = -1.0, p < .0001$). There were also perfect positive correlations between prevalence of subsyndromal SAD and latitude in both doctors' offices and the general population ($r = 1.0, p < .0001$). However, while the correlation between latitude and the prevalence of winter SAD was not significant for doctors' offices ($r = 0.5, p = .667$), a perfect correlation was found in the general population. The lack of correlation between prevalence of winter SAD and latitude in doctors' offices may be due to the fact that no data was collected from these offices during the high risk months of January and February when most winter seasonals become symptomatic and are likely to seek treatment. For the same reason it is impossible for us to demonstrate that winter seasonals attend doctors' offices in the winter months at the more northern locations. However, the lower prevalence of winter SAD in Nashua doctors' offices during the summer and fall suggest that winter seasonals in Nashua may be less likely to go to doctors in those particular months. We were able to demonstrate that summer seasonals in Sarasota are most likely to attend doctors' offices in the month of July ($p < .05$).

Patients in D.C. and Sarasota had significantly higher Global Seasonality Scores (GSS) than the non-patient responders, but this difference was not found in Nashua. In general, patients reported significantly more favorable responses to cold weather and significantly less favorable responses to hot weather than non-patients ($p < .05$).

Significance to Biomedical Research:

The series of epidemiological studies discussed above have served to define the scope of the problem which seasonal changes present to the general population. It appears that approximately one-fifth of the adult population of the U.S. suffers from these changes. If criteria derived from the SPAQ for the diagnosis of SAD and S-SAD are accurate, and if the sites evaluated in our study are representative of the U.S. as a whole, prevalence rates of these two conditions are estimated at 6.1% and 14.3% respectively. However, these figures need to be confirmed by systematic epidemiological studies, in which more subjects are evaluated clinically.

Until such time, however, they are our best estimates of the prevalence of these conditions and they corroborate clinical and anecdotal impressions that seasonal problems in mood and behavior are not uncommon. Prevalence rates for SAD and S-SAD have important public health implications inasmuch as these conditions impair quality of life, reduce productivity, and are potentially reversible by modification of environmental lighting. We have explored this last point by evaluating the effects of bright light exposure in a subgroup of respondents in the Montgomery County study in an attempt to determine what proportion of the general population stands to benefit in some way from phototherapy (see Annual Report #ZO1 MH 02205).

Proposed Course:

We are currently collaborating on a study titled "Seasons Around the World" under the auspices of Dr. Lennart Wetterberg at the Karolinska Institute in Sweden. As part of this study, normal subjects at approximately 30 different sites around the globe have filled out the Seasonal Pattern Assessment Questionnaire, which will provide the first estimate of worldwide seasonal patterns. Several groups in the United States and Europe have expressed interest in using the SPAQ to survey the prevalence of seasonal changes in their geographical locations.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02423-01 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Causes and Treatments of Rapid Cycling Affective Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH
Others:	N. E. Rosenthal	Chief, Unit on Outpatient Studies	CPB/NIMH
	D. A. Sack	Chief, Unit on Inpatients Studies	CPB/NIMH
	R. W. Cowdry	Deputy Director	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

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- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Affective illness tends to remit and recur spontaneously and with increasing frequency. Its course is also characterized by a tendency for manic episodes to be immediately preceded or followed by depressive episodes, with no intervening normal period, and for mania to alternate with depression. In rapid cycling affective disorder these tendencies are so pronounced that mania and depression recur regularly and frequently with a continuous, circular course. Rapid cycling cases comprise about 15% of patients in lithium or affective disorder clinics, and they are difficult to treat. The purpose of this project was to gain further insights into the causes and treatments of rapid cycling affective disorder by comparing data obtained from rapid cycling and non-rapid cycling patients with regard to their clinical features and course of illness.

All rapid cycling patients admitted to our research program since 1973 were included in the study. For comparison, a non-rapid cycling control group was also investigated. Information regarding the patients' psychiatric and medical histories, family histories, course of illness, and responses to treatments was derived from hospital charts, research flow charts containing records of prospective daily mood ratings, treatments and procedures, and structured follow-up interviews.

We found that 1) rapid cycling affective disorder is phenotypically and genetically related to more typical forms of bipolar affective disorder; 2) there was a high prevalence of thyroid disease during lithium treatment in both rapid cycling and non-rapid cycling women; 3) although nearly all the rapid cycling patients were women, there was no convincing evidence that the rapid cycles were generated by the menstrual cycle; 4) treatment with antidepressant drugs was associated with reversible rapid cycling in approximately 50% of the rapid cycling patients; patients experienced slowing or cessation of cycling when the drugs were withdrawn.

The observation that antidepressants may have been responsible for rapid cycling in half the cases has obvious implications for prevention and treatment of rapid cycling, and it provides a clue to possible neurochemical causes of rapid cycling.

Project Description:

One of the distinguishing features of affective illness is its tendency to remit and recur spontaneously and with increasing frequency. The course of bipolar illness is also characterized by a tendency for manic episodes to be immediately preceded or followed by depressive episodes, with no intervening normal period, and for mania to alternate with depression. In rapid cycling affective disorder these tendencies are so pronounced that mania and depression recur regularly and frequently with a continuous, circular course. Rapid cycling cases comprise about 15% of patients in lithium or affective disorder clinics, and they are difficult to treat.

There has been some progress in elucidating the possible causes of rapid cycling. Wehr and Goodwin found that antidepressants are capable of causing rapid cycling in bipolar patients who were not previously rapid cyclers. Also, thyroid disease has been implicated in rapid cycling affective disorder, and women seem to be particularly susceptible to this form of affective illness.

The purpose of this project was to gain further insights into the causes and treatments of rapid cycling affective disorder by comparing data obtained from rapid cycling and non-rapid cycling patients with regard to their clinical features and course of illness.

Methods:

All patients who were admitted to our research program since 1973 with a clear-cut history of rapid cycling affective disorder were included in the study. For purposes of comparison, a non-rapid cycling control group was also investigated. The diagnosis of major affective disorder was made by two psychiatrists using Research Diagnostic Criteria (RDC). Rapid cycling was defined as four or more affective episodes per year that, in bipolar patients, followed a circular course at some time in the history of the illness. Information regarding the patients' psychiatric and medical histories, family histories, course of illness, and responses to treatments was derived from four sources: the investigators' firsthand knowledge of each patient; standard hospital charts; research flow charts containing records of prospective daily mood ratings, treatments and procedures; and in 94% of the cases, a structured follow-up interview conducted by one of the investigators.

Findings to Date:

Fifty-one patients with rapid cycling affective disorder were admitted to our research program since 1973. All were classified as having bipolar affective disorder and all met RDC criteria for endogenous depression. The control group consisted of 19 women with non-rapid cycling bipolar illness who were admitted during the same period.

Several of the findings in this study suggest that rapid cycling affective disorder is phenotypically and genetically related to more typical forms of bipolar affective disorder. First, all the rapid cycling patients met the RDC for bipolar affective disorder. Second, the ages at onset of affective illness in the rapid cycling patients were strikingly similar to those reported for non-rapid cycling bipolar patients. Third, in more than half the cases, patients who ultimately developed rapid cycling began their illness with a pattern of occasional, isolated episodes that resembled the typical course of illness in non-rapid cycling bipolar patients. Fourth, family histories revealed a high genetic loading for non-rapid cycling unipolar and bipolar affective disorder, similar to that seen in non-rapid cycling cases, and a small familial tendency for rapid cycling.

There was a high prevalence of thyroid disease during lithium treatment in both rapid cycling and non-rapid cycling women. These findings are consistent with other reports of a high prevalence of hypothyroidism in women over 40 years of age who have been treated with lithium. These findings contrast with earlier reports of a higher prevalence of thyroid disease in rapid cycling than in non-rapid cycling patients. The high prevalence of thyroid disease in our study may simply reflect the fact that most of the cases involved women, that thyroid disease is much more common in women than in men, and that most of the patients had been treated with lithium, a known thyrotoxin.

Ninety-two percent of the rapid cycling patients were women, a finding that is consistent with trends observed in other studies. Although nearly all the rapid cycling patients were women, there was no convincing evidence that the rapid cycles were generated by the menstrual cycle.

By history, treatment with antidepressant drugs was associated with reversible rapid cycling in approximately 50% of the rapid cycling patients; patients experienced slowing or cessation of cycling when the drugs were withdrawn. Prospective, longitudinal observations of 30% of the patients confirmed the association between antidepressants and rapid cycling.

Withdrawal of antidepressants seemed to be a useful approach to the treatment of a significant number of rapid cycling patients. Fourteen percent of the fifty-one patients eventually stabilized after withdrawal of antidepressants while they continued to be treated with lithium carbonate alone. In some patients who ultimately responded to treatment with lithium alone, lithium had been ineffective in treating antidepressant-induced rapid cycling.

Significance to Biomedical Research:

The results of this study provide some insights into the causes of frequent recurrences of affective illness, and can be used to improve the treatment of rapid cycling patients. First, that fact that women are much more susceptible to rapid cycling forms of affective illness is an important clue to its causation. Although the incidence of thyroid disease is no more common in rapid cycling patients than in other groups of women who have been exposed to lithium, women's vulnerability to thyroid disease might nevertheless be related to their vulnerability to rapid cycling. The observation that rapid mood cycles were not synchronized with menstrual cycles suggests that the manic-depressive cycles are not driven by the menstrual cycle and therefore that treatment strategies aimed at suppression of the menstrual cycle would not necessarily be likely to suppress the manic-depressive cycles. The observation that antidepressants may have been responsible for rapid cycling in half the cases has obvious implications for prevention and treatment of rapid cycling, and it provides a clue to possible neurochemical causes of rapid cycling.

Proposed Course:

This project was designed to review and synthesize the results of fourteen years of longitudinal investigations of rapid cycling affective disorder. This process has been completed and the project is terminated.

Publications

Wehr TA, Goodwin FK. Can antidepressants cause mania and worsen the course of affective illness? *American Journal of Psychiatry* 1987;144:1403-1411.

Wehr TA, Sack DA, Rosenthal NE, Cowdry RW. Rapid cycling affective disorder: Contributing factors and treatment responses in 51 cases. *American Journal of Psychiatry* 1988;145:179-184.

Wehr TA, Goodwin FK. Do antidepressants cause mania? *Psychopharmacology Bulletin* 1987;23:61-65.

Wehr TA, Sack DA, Rosenthal NE. Sleep and biological rhythms in bipolar illness, in Goodwin FK, Jamison K (eds): *Bipolar Disorders*, in Hales RE, Frances AJ (eds): *American Psychiatric Association Annual Review: Volume 6, 1987*, American Psychiatric Press, Washington D.C., pp. 61-80.

Wehr TA. Causes and treatments of rapid cycling affective disorder, in Amsterdam JD (ed), *Depression Treatment*, New York, Marcel Dekker, Inc., in press.

Z01 MH 02424-01 CP

October 1, 1987 to September 30, 1988

Biological Mechanisms of the Antidepressant Effects of Sleep Deprivation

PI: T. A. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

Others:	N. E. Rosenthal	Chief, Unit on Outpatient Studies	CPB/NIMH
	P. Schulz	Social Worker	CPB/NIMH
	S. Kasper	Visiting Associate	CPB/NIMH
	K. Kelly	Medical Staff Fellow	CPB/NIMH
	J.R. Joseph-Vanderpool	Medical Staff Fellow	CPB/NIMH

COOPERATING UNITS (if any)

Clinical Psychobiology Branch

SECTION

NIMH, Bethesda, Maryland 20892

OTHER:

2

1

1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Total sleep deprivation for one night induces temporary remissions in sixty percent of patients with major depression; it can also induce mania in bipolar patients. These observations have practical implications for the management of affective illness. For example, some patients' depressions can be treated with sleep deprivation, and sleep disruption is sometimes an identifiable and preventable cause of mania. Knowledge of biological mechanisms of sleep deprivation would undoubtedly increase our understanding of the causes of depression and mania and lead to new types of drug treatments for depression and mania. The purpose of this project is to identify biological mechanisms of the antidepressant effects of sleep deprivation by 1) determining those aspects of the sleep deprivation procedure which are responsible for its antidepressant effects, and 2) investigating the effects of sleep deprivation on biological variables, such as hormones and neurotransmitter metabolites, which might mediate its antidepressant effects.

We have already determined that 1) exposure to light at night is not necessary for the antidepressant effects of the procedure and 2) partial sleep deprivation in the second half of the night is much more effective than partial sleep deprivation in the first half of the night.

The observation that sleep deprivation increases plasma levels of thyrotropin (TSH) is of particular interest because nocturnal levels of TSH are deficient in depressed patients. The effects of sleep deprivation on TSH resemble effects of cold exposure. Therefore, in an ongoing experiment we are testing the hypothesis that the effects of sleep deprivation on mood and on TSH secretion in depressed patients depend on heat loss from the organism. According to this hypothesis sleep deprivation in a warm environment should be less effective than sleep deprivation in a cold environment. Patients and normal individuals are sleep deprived on one occasion in a very warm environment (90° F), on another occasion in a very cool environment (62° F). During each sleep deprivation, clinical state, body temperature, and hormone secretion are assessed. So far, we have found that heating of the environment increases rectal temperature and decreases TSH levels. In the patients, antidepressant effects of sleep deprivation are greater in the cool condition than in the warm condition. So far, these results are consistent with predictions based on our hypothesis, but several additional subjects are needed to permit statistical evaluation of the results.

Project Description:

Total sleep deprivation for one night induces temporary remissions in sixty percent of patients with major depression. Like other antidepressant agents, sleep deprivation can also induce mania in bipolar patients. These observations have practical implications for the management of affective illness. For example, some patients' depressions can be treated with sleep deprivation. Unfortunately, however, many patients relapse during recovery sleep after they have responded to sleep deprivation. In these cases repeated partial sleep deprivation or coadministration of drugs may help to sustain the antidepressant response to the procedure. In the course of bipolar illness, emotional states and life events which disrupt sleep may cause mania through the sleep deprivation mechanism. In many cases these factors are identifiable and preventable causes of mania. The effects of sleep and sleep deprivation on affective illness are also important clues to the biological mechanisms which cause depression and mania. Stated simply, a process connected with wakefulness is capable of improving depression and causing mania, and a process connected with sleep is capable of improving mania and causing depression (at least in patients who relapse during recovery sleep). Clearly, if we had more knowledge of these biological mechanisms, we would understand more about the causes of depression and mania. Knowledge of biological mechanisms of sleep deprivation would undoubtedly suggest radically new types of pharmacological treatments for depression and mania. Drugs which mimic or block the effects of sleep deprivation might be used to treat depression and mania, respectively, and, like sleep deprivation, they might be expected to act much more rapidly than currently available drugs. The purpose of this project is to identify biological mechanisms of the antidepressant effects of sleep deprivation by 1) determining those aspects of the sleep deprivation procedure which are responsible for its antidepressant effects, and 2) investigating the effects of sleep deprivation on biological variables, such as hormones and neurotransmitter metabolites, which might mediate its antidepressant effects. We have already made some progress in both of these areas.

Aspects of the sleep deprivation procedure which are responsible for its antidepressant effects. The procedure that we call "sleep deprivation" is complex and consists of many simultaneous interventions. When they are sleep deprived, patients are also subjected to changes in posture, ambient temperature, light, diet, social contact, etc. In principle, any of these changes might be responsible for the antidepressant effects of the procedure. It is even conceivable that sleep deprivation per se is not responsible. In sleep deprivation experiments with depressed patients we have determined that 1) exposure to light at night is not necessary for the antidepressant effects of the procedure and 2) partial sleep deprivation in the second half of the night is much more effective than partial sleep deprivation in the first half of the night. This latter finding suggests that the antidepressant effects of the procedure may depend on interactions of sleeping and waking with a process governed or gaited by a circadian rhythm.

Effects of sleep deprivation on biological variables. In a series of experiments our group and others have investigated the effects of sleep deprivation on a variety of hormones and neurotransmitter metabolites which might mediate its effects on clinical state in affective illness. Sleep deprivation has little effect on melatonin secretion by the pineal gland, or on plasma levels of homovanillic acid (HVA) and 3-methoxy, 4-hydroxyphenylglycol (MHPG), metabolites of the neurotransmitters, dopamine and norepinephrine. Sleep deprivation increases plasma levels of thyrotropin (TSH) and cortisol and decreases plasma levels of prolactin (PRL) and growth hormone (GH). The effects of sleep deprivation on TSH are of particular interest because our previous research, as well as that of other groups, has shown that nocturnal levels of TSH are deficient in depressed patients. Thus, sleep deprivation, which normalizes mood in depressed patients, also tends to normalize their low nocturnal levels of TSH. Our current research on possible mechanisms of the antidepressant effects of sleep deprivation is focused on the restoration of normal TSH secretion by the procedure.

The surge in TSH secretion which normally occurs at night can be completely blocked by external heating with an electric blanket. In fact, other experiments have shown that heat suppresses and cold stimulates TSH secretion. In contrast heat stimulates and cold suppresses PRL secretion. Thus, the effects of sleep deprivation on these two hormones resemble effects of cold exposure. In an ongoing experiment we are testing the hypothesis that the effects of sleep deprivation on mood and on TSH and PRL secretion in depressed patients depend on heat loss from the organism. According to this hypothesis sleep deprivation in a warm environment should be less effective than sleep deprivation in a cold environment.

Methods:

Patients and normal individuals are sleep deprived for one night on two different occasions. On one occasion they are sleep deprived in a very warm environment (90° F), on another occasion they are

sleep deprived in a very cool environment (62° F). During a 24 hour period with sleep, and during the two 24 hour periods of sleep deprivation in warm and cool environments, clinical state, body temperature, and hormone secretion are assessed. Before and after sleep deprivation, raters who are blind to the sleep deprivation conditions evaluate mood with the Hamilton Rating Scale for Depression. Patients also evaluate themselves with analogue mood self-rating scales. For 24 hours in each of the three conditions, blood samples are drawn every hour and subsequently analyzed for plasma levels of TSH, PRL, T3, T4, Free T3, Free T4, melatonin and cortisol.

Findings to Date:

So far, three depressed patients and three normal individuals have completed the study. In these subjects, heating of the environment increased rectal temperature, increased PRL levels, and decreased TSH levels. In the patients, antidepressant effects of sleep deprivation were greater in the cool condition than in the warm condition. All of these results are consistent with predictions based on our hypothesis. However, the small number of subjects is small. Several additional subjects are needed to permit statistical evaluation of the results.

Significance to Biomedical Research:

Our research suggests that thermoregulatory mechanisms may play an important role in the pathophysiology of depression and mania, and in the antidepressant effects of sleep deprivation. If true, this conclusion would establish an unexpected link between the effects of sleep deprivation and the effects of seasons on mood. In summer depressives, results of preliminary experiments (Z01 MH) suggest that heat may induce and cold may improve depressive symptoms. Identification of biological mechanisms of the antidepressant effects of sleep deprivation undoubtedly would lead to the development of efficient new drug treatments for depression and mania.

Proposed Course:

We hope to complete this difficult, time-consuming experiment during the coming year. If preliminary results are confirmed in additional subjects, our subsequent research will focus increasingly on the possible role of thermoregulatory mechanisms in the pathogenesis and treatment of depression and mania.

Publications:

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02425-01 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Thermoregulatory Functions of Slow-Wave Sleep and REM Sleep

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. A. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

Others: E. Souetre Visiting Scientist CPB/NIMH
J.R. Joseph-Vanderpool Medical Staff Fellow CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Section on Sleep Studies

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to investigate basic biological functions of sleep and sleep stages. Sleep, like torpor, may serve to conserve energy by inhibiting behavioral responses to environmental stimuli and by lowering the regulated level of body temperature and metabolism. Such repeated small savings in energy expenditure could enhance an animal's chance of survival in environments with marginal energy resources. Most investigators have focused on energy conserving aspects of slow wave sleep, which appears to be associated with a temperature-lowering mechanism.

Current dogma is that thermoregulation is suspended during REM sleep. However, considerable energy is expended by the brain during REM sleep, and it seems unlikely that this expenditure serves no useful purpose. We hypothesize that REM sleep serves to generate heat locally in the brain and the eyes in order to maintain CNS temperature within acceptable limits while the rest of the body cools during sleep. Heat production is accomplished by 1) increased brain metabolism and 2) rapid eye movements. Maintenance of brain temperature may 1) facilitate rapid arousal from sleep and/or 2) protect CNS tissue in homeotherms from functional or structural impairment during the considerable body cooling which occurs when they sleep in a natural environment.

The purpose of this project is 1) to document changes in somatic and CNS temperature during the different stages of sleep by measuring sleep EEG and rectal, chest, finger, eyelid, forehead and tympanic membrane temperatures continuously during sleep, and during voluntary rapid eye movements during wakefulness and 2) to test the hypothesis that REM sleep maintains brain temperature during body cooling by testing the response of REM sleep generating mechanisms to thermal challenges administered to the sleeping brain. During slow wave sleep, we found that rectal, forehead, eyelid and tympanic temperatures decline rapidly. During the first REM sleep episode and during voluntary rapid eye movements during wakefulness, we found that rectal temperature (a measure of somatic temperature) continues to decline, but that forehead, eyelid, and tympanic temperatures (indirect measures of CNS temperature) rise. These descriptive observations are in accord with our model of selective brain thermogenesis during REM sleep.

We have begun to study the effects of facial heating and cooling on REM sleep generating mechanisms. Data from these experiments have not yet been analyzed.

Project Description:

Little is known of the biological functions of sleep and of the stages of sleep, such as slow-wave sleep and REM sleep. There is some agreement that sleep may serve to conserve energy, in a manner analogous to torpor, by inhibiting behavioral responses to environmental stimuli and by lowering the regulated level of body temperature and metabolism. In a natural environment such repeated small savings in energy expenditure could enhance an animal's chances of survival in environments with marginal energy resources. Work of other investigators has focused mainly on energy conserving aspects of slow wave sleep. Slow wave sleep appears to occur in association with a thermolytic, or temperature-lowering mechanism. Animals enter hibernation (a state of profound hypothermia) through slow-wave sleep, and, in human beings, heating of the head during sleep induces slow-wave sleep, even at the end of the sleep period when it is normally absent.

There has been little investigation of the possible thermoregulatory functions of REM sleep. In fact, the current dogma is that thermoregulation is suspended during REM sleep, rendering mammals and birds poichilothermic during this stage of sleep. Considerable energy is expended by the brain during REM sleep, and it seems unlikely that this expenditure serves no useful purpose. We hypothesize that the function of the marked increase in energy expenditure by the central nervous system during REM sleep is to generate heat locally in the brain and the eyes in order to maintain CNS temperature within acceptable limits while the rest of the body cools during sleep. Thus, according to this hypothesis, REM sleep is a form of selective brain thermogenesis. CNS thermogenesis during REM sleep is accomplished through 1) increased metabolism and 2) increased eye muscle tone and movement. Heat generated by eye movements, in addition to heating the eyes, may also augment heating of pituitary and brain structures by convection via venous drainage into the cavernous sinus. Ultimately, the purpose of brain temperature maintenance during sleep may be 1) to facilitate rapid arousal from sleep and/or 2) to protect CNS tissue in homeotherms from functional or structural impairment during the considerable body cooling which occurs when they sleep in a natural environment (in aborigines sleeping naked out of doors, for example, rectal temperature declines to 33° C).

There are many connections between homeothermic temperature regulation and REM sleep. For one thing, REM sleep probably only occurs in homeotherms and has co-evolved with homeothermy independently in mammals and birds. The duration of the REM non-REM cycle is highly correlated with an animal's size, or more specifically, with the animal's surface area to mass ratio, which determines the rate at which heat is lost from the body. There is a close, inverse relationship between REM propensity and the daily rhythm in body temperature; REM sleep is greatest when body temperature reaches its minimum at the end of the sleep period. During development, the amount of REM sleep is greatest at the beginning of life and declines as somatic thermoregulatory mechanisms mature.

The purpose of this project is 1) to document changes in somatic and CNS temperature during the different stages of sleep and 2) to test the hypothesis that REM sleep maintains brain temperature during body cooling by testing the response of REM sleep generating mechanisms to thermal challenges administered to the sleeping brain.

Methods:

In the first phase of this project we have measured sleep EEG and rectal, chest, finger, eyelid, forehead and tympanic membrane temperatures continuously during sleep, and during voluntary rapid eye movements during wakefulness.

In a second phase of this project we have begun to measure the effects on sleep stages of facial and/or whole body heating and cooling prior to and/or during sleep.

Findings to Date:

During slow wave sleep, rectal, forehead, eyelid and tympanic temperatures decline rapidly. During the first REM sleep episode, rectal temperature (a measure of somatic temperature) continues to decline, but forehead, eyelid, and tympanic temperatures (indirect measures of CNS temperature) rise until REM sleep is terminated. During voluntary rapid eye movements in awake subjects, forehead, eyelid and tympanic temperatures rise, as occurs in REM sleep. All of these

descriptive observations are in accord with our model of selective brain thermogenesis during REM sleep.

We have begun to study the effects of facial heating and cooling on REM sleep generating mechanisms. Data from these experiments have not yet been analyzed.

Significance to Biomedical Research:

To identify a basic physiological function of REM sleep would be a fundamental contribution to our knowledge of biology and medicine. Within the framework of the research conducted by this branch, any knowledge about biological mechanisms responsible for REM sleep would be relevant to the basic mission of investigating causes and developing new treatments for affective illness. There are many important connections between REM sleep and depression. Extensive research has shown that certain manifestations of REM sleep are excessive in depressed patients, and most effective treatments for depression suppress these manifestations of REM sleep. Other evidence indicates that temperatures during nocturnal sleep are abnormally elevated in depressed patients, and that these elevations may be responsible for some of the endocrine abnormalities observed in depression. Our sleep deprivation experiments (Z01 MH) and our studies of seasonal influences on depression (Z01 MH) suggest that excessive heating may induce depressive symptoms. If our hypothesis is correct that REM sleep supports CNS thermogenesis, then its putative capacity to trigger depression might be related to its capacity to heat the brain.

Proposed Course:

Our current investigations of the effects of heating and cooling on REM sleep generating mechanisms will continue during the coming year.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 MH 02426-01 CP

PERIOD COVERED

February 1, 1988 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiology of Sleep and Sleep Loss

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Everson Research Fellow CPB/NIMH

Others: T. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Section on Sleep Studies

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

1

OTHER:

.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This is a prospective overview of new avenues of animal research in sleep physiology in the Clinical Psychobiology Branch. Our experimental aim is to investigate unresolved, basic sleep research issues. During the last four months, we have formulated the issues which we will address as well as the experimental designs. We are currently making good progress in devising and allocating equipment necessary to carry out the experiments.

The function(s) of sleep remain unknown, even though sleep as a phenomenon has been well described. The long-term goal of our sleep research is to find out what is accomplished physiologically during sleep that cannot be accomplished during wakefulness or rest. Our working assumption is that the limits to the biological significance of sleep are unknown.

Below are three initial strategies we will use to study sleep:

1. To remove sleep and note the resulting impairments. By searching for intermediary processes which underlie consequences of sleep loss we may be able to infer a function normally fulfilled by sleep. The aim of Study 1 is to determine which sleep loss symptoms in the rat indicate a physiological system primarily affected by deprivation, and which symptoms are secondary effects.

2. To search for functional relationships between two processes which appear to co-vary, such as alterations in sleep and energy balance. In Study 2, the metabolic rate of rats will be manipulated via the thyroid system to determine whether sleep amount is lawfully related to changes in metabolic status.

3. To search for intermediary effects of variables which markedly alter normal sleep parameters. Study 3 will investigate a previous report that sleep time is dramatically increased by lack of two specific, essential fatty acids. In Study 4, we will assess whether the sleep-inducing effects of prostaglandins might be due to changes in core or brain temperature.

Project Descriptions And Methods:

STUDY 1-- PHYSIOLOGY OF SLEEP LOSS.

Background. The first step in studying sleep loss in rats was to profile biochemical and physiological changes after long-term deprivation. Everson et al.^{1,2} found that chronic sleep loss resulted in a specific syndrome, rather than a generalized impairment of all physiological systems. Sleep loss symptoms can be grouped under five main deprivation effects:

1) **Mortality.** Totally sleep deprived (TSD) rats died, or were sacrificed when death appeared imminent, after 20.9 (SD = 5.9) days of sleep loss. Yoked control (TSC) rats appeared as though they could continue living under the experimental conditions. Since sleep is indispensable, it must serve a fundamental biological purpose.

(Some results for TSD rats will be described in terms of quarters of survival time. Each rat's survival time was divided into quarters and data were averaged for each quarterly bin, first within rats and then across rats.)

2) **A progressive increase in food intake concomitant with body weight loss.** Daily food intake eventually reached 80% above baseline values during the last quarter of survival. Mean weight loss from baseline during the same period was 12%. This catabolic state could not be explained by malabsorption of nutrients, hyperactivity, diabetes, or dehydration. Indirect calorimetric measurements showed a near two-fold increase in energy expenditure.

3) **Protein-related deficits.** In spite of increased protein ingestion, plasma albumin declined progressively after the first quarter of survival, eventually reaching an average of 30% below baseline levels during the last quarter of survival. We could not find a cause of the hypoalbuminism: a) protein was not lost in the urine, b) rats did not suffer from malabsorption, and c) liver dysfunction was not indicated in the results of assays of liver enzymes or globulin electrophoresis.

During experimental quarters three and four, TSD rats developed a normocytic anemia. The anemia was characterized by a decline in total red blood cell mass without significant replacement by immature cells; a profile similar to that found in protein malnutrition states. The anemia was most likely not caused by blood loss because hemorrhages were not seen on organs at necropsy, and patterns of mild urinary blood in both TSD and TSC rats did not parallel the developing anemia. Red blood cells were not excessively destroyed; plasma levels of conjugated and unconjugated bilirubin and the liver enzyme, GTT, remained stable and normal.

4) **Severe stereotypic ulcerative and hyperkeratotic skin lesions** located on the tail and plantar surfaces of the paws. Necrotizing vasculitis, trauma, and zinc deficiency were ruled out as causes of the lesions.

5) **Body temperature changes.** A mild increase (0.5 °C) in core temperature was concomitant with the raised energy expenditure during the first half of the experimental period. During the second half of the experimental period, core temperature declined below baseline levels, indicating a difficulty retaining body heat.

Histological examination of organs, including brain, of other TSD rats deprived by the same methods were negative.³

Project description:

Our first experimental aim is to search for the most fundamental deprivation-induced pathology. We will begin by attempting to separate the effects of a protein-related deficiency from those of increased energy expenditure. If sleep serves to promote synthetic activities, as some researchers have proposed,⁴ a protein deficiency would be an expected outcome of sleep loss. Physiologic attempts to off-set increased protein degradation, impaired synthetic processes, or rid nonprotein calories could have raised energy expenditure. On the other hand, increased energy expenditure from a different origin may have caused excessive protein metabolism, resulting in a protein deficiency.

The second aim is to determine whether a protein deficiency might explain several sleep loss symptoms, regardless of whether the protein loss was secondary to increased energy expenditure. Many symptoms of TSD rats resemble those of Kwashiorkor patients, who ingest few proteins but relatively normal calories: hyperkeratotic skin lesions with superficial ulceration, mild to severe edema, low hemoglobin, suppressed circulating lymphocytes, hypoalbuminism, normal total plasma proteins, and hair changes;⁵ all symptoms which were seen in TSD rats.

Procedure. To deprive rats of sleep, we will employ the same procedures used in the experiments discussed above. The procedures were developed and refined by Drs. Allan Rechtschaffen and Bernard Bergmann of the University of Chicago Sleep Research Laboratory. Confounds inherent in other experimental designs were eliminated by employing two important controls for the procedures used to maintain prolonged sleep deprivation: a) use of a benign arousal stimulus, and b) yoked controls which obtained most of their baseline sleep amounts:

Each TSD rat was housed opposite to a TSC rat on a divided platform suspended over shallow pans of water. When sleep onset was detected by changes of EEG activity of the TSD rat, the platform was automatically rotated and both rats were required to walk in a direction opposite to the rotation to avoid being forced into the water. The rotation was slow and ended when the TSD rat was awake. In this manner, forced locomotion was minimized and applied to both rats simultaneously. TSC rats could sleep whenever the disk was stationary.

It has been shown that rats increase food intake to compensate for both protein and caloric deficiencies.⁶ Therefore, we can manipulate the diet to make it easier for TSD rats to meet either a high caloric need or a high protein need. TSD-TSC pairs will be given either a calorically dense diet (with the same amount of proteins as a normal diet) or a protein dense diet (isocaloric to a normal diet). If TSD rats on a high protein diet (TSD-HP) consume the same amount (in grams) or less than TSD rats on a normal diet (TSD-N), yet have symptoms reduced in severity or eliminated, the evidence would support the hypothesis of an increased need for proteins during sleep loss. If the TSD-HP group ingests the same amount as the TSD-N group but does not show improvement in symptoms, protein loss probably did not mediate the pathologies. If TSD rats on the high caloric diet (TSD-HC) consume the same number of grams or less than the TSD-N group and have reduced pathology, they must have been able to better meet high energy needs. Differential symptom profiles in TSD rats under the two dietary manipulation paradigms will afford an assessment of the relative contributions of calories and proteins to the pathology of sleep loss.

To further assess whether ingested proteins are used for synthetic activities during sleep loss, we will employ a method of *in vivo* protein labeling. We are currently evaluating the feasibility of various labeling methods with the sleep deprivation protocol. Whole body protein incorporation appears to be the best method for evaluating whether proteins are used for synthetic activities during sleep loss. Outcomes of other methods, such as measurements of plasma protein turnover, would be inconclusive because TSD rats are hypermetabolic and increased turnover would be expected.

Rats will be surgically prepared for chronic EEG recordings. Measurements will include food and water intake, body weights, core and brain temperature, and descriptions of skin pathology. Some rats will be cannulated for daily drawing of blood samples for clinical chemistry and hematology analyses.

STUDY 2-- THE INTERACTION BETWEEN SLEEP DURATION AND ENERGY EXPENDITURE.

Project description:

Several lines of evidence suggest a strong positive relationship between metabolic rate and sleep. For example, sleep duration is highly correlated with estimates of metabolic rate across species; young animals spend a greater proportion of their time asleep than adults; in humans, the higher the waking body temperature (which correlates positively with metabolic rate), the greater the duration

of sleep; a near doubling of metabolic rate occurs in rats during long-term sleep deprivation. If sleep and energy balance are intricately related, sleep duration should be sensitive to states of increased or decreased energy utilization.

In this study, we will monitor changes in sleep parameters during manipulations of metabolic rate. Rats will be made hypometabolic by chemical thyroidectomy (oral propylthiouracil) or by blocking the 5'-monodeiodination of thyroxine (T_4) to triiodothyronine (T_3 ; the most metabolically active form of T_4). Hypothyroid rats will be made euthyroid or hyperthyroid by administration of different doses of T_3 . We chose T_3 , rather than T_4 , administration to avoid T_4 disposal through metabolically inactive routes, and to increase the endogenous ratio of T_3 to T_4 , as in cold exposed and hypercaloric states (i.e., two comparison groups of altered energy balance). If sleep is sensitive to changes in energy balance, a difference should be apparent between the sleep durations of the hypometabolic and hypermetabolic states.

Procedure:

Rats will be surgically prepared for chronic recording of EEG activity. A thermistor and telemetric transmitter will be implanted for recording of brain and core temperatures respectively. If plasma T_4 assays seem warranted, some rats will be implanted with jugular catheters for daily blood drawing.

A long electrode recording cable and commutator will allow continuous recording of EEG activity and unrestricted movement of the rats within their cages. Food and water intake, body weights, and core and brain temperature will be monitored. Cage temperature and light schedules may be manipulated during baseline and experimental conditions as biological probes on sleep parameters.

Rats in the chemical thyroidectomy group will receive propylthiouracil in their diet. For other manipulations of the thyroid system, drugs will be administered parenterally.

STUDY 3-- SLEEP EFFECTS OF ESSENTIAL FATTY ACID DEFICIENCY.

Project description:

Essential fatty acid deficiency (EFAD) in rats produced a near 50% increase in total sleep time.⁷ Physiologic mediation of this dramatic effect was not pursued, possibly because the sleep effect was not the main focus of the study. This is a rare situation in which withholding, rather than administration, of a relatively specific substance alters sleep parameters.

In this study, we will search for the mechanisms of the sleep-inducing effect in the EFAD state. Several important questions will be addressed:

1. Are there qualitative differences between sleep in normal and EFAD rats? For example, do EFAD rats sleep longer because their sleep is less intense (i.e., as measured by EEG frequency and amplitude analysis)?
2. Might changes in brain myelination be the cause of changes in the qualitative aspects in sleep of EFAD rats?
3. Are changes in core and brain temperature during sleep in EFAD rats similar to those seen during sleep in normal rats? Are indications of an altered energy balance reflected in the temperature measurements during sleep?
4. Which essential fatty acids are necessary to restore normal sleep? Will end products of fatty acid synthesis (e.g., prostaglandins) normalize sleep in EFAD rats?

Procedure:

To make an EFAD diet, coconut oil or lard will be substituted for the usual unsaturated oils in a purified diet. The diet will also contain an indigestible material which will pass into the feces and can be used to calculate an index of energy efficiency.

Other experimental conditions will be similar to those described in Study 2.

STUDY 4-- EXAMINATION OF PROSTAGLANDIN-INDUCED SLEEP.

Project description:

Prostaglandins (PGs) are potent, putative sleep-inducing substances. Mechanisms which underlie the PG effect on sleep are unknown. PGs also influence core, and possibly brain, temperature. Sleep and typical patterns of sleep stage alterations are associated with changes in core and brain temperature. Do PGs exert their effect on sleep because they alter core and brain temperature?

This study will search for mediation of the PG effect on sleep, starting with an examination of the correspondence between PG-induced changes in core and brain temperature, and changes in sleep parameters.

Procedure:

Experimental conditions will be essentially the same as those of Study 2.

Administration of PG-related substances will be given via osmotic mini pumps, venous catheter, or i.p. injection, depending on the drug vehicle and characteristics of drug metabolism.

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4. Adam K, Oswald I. J Roy Coll Physcns 1977;11:376-388.
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SIGNIFICANCE TO BIOMEDICAL RESEARCH

Outcomes of our investigations of sleep physiology will provide basic biological knowledge about a phenomenon that is poorly understood. Furthermore, functional studies such as these provide direction for reductionist approaches (e.g., neuro- and molecular biology) in sleep research.

Companion reports from the Clinical Psychobiology Branch, by Dr. Thomas Wehr and colleagues, point to the possible implications of sleep dysfunction in psychiatric disorders, such as depression. Some depressed patients derive immediate benefit from sleep deprivation. Outcomes of animal experiments on the physiology of sleep and sleep loss may help elucidate the role of sleep in psychiatric pathology.

In general medicine, the more we know about processes which underlie sleep, the sooner we will be able to determine a role for sleep in clinical, nonpsychiatric pathologies. How might subtle changes in sleep be related to etiologies of disease processes? Does sleep really help recovery from illness? If so, how might sleep be manipulated to aid recovery from disease?

Proposed Course:

We will proceed first with Studies 1 (Physiology of Sleep Loss) and 3 (Sleep Effects of Essential Fatty Acid Deficiency). These two studies are the least preliminary and the outcomes can potentially yield the most specific information about physiological processes involved in sleep or sleep loss. As we get more equipment and resources in place, we will conduct Studies 2 and 4.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02427-01 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Circadian Regulation of Intergeniculate Leaflet Neuropeptide Y Messenger RNA in Syrian Hamsters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. C. Duncan	Research Psychologist	CPB/NIMH
Others:	E. Sutin	Biologist	CPB/NIMH
	D. Jacobowitz	Chief, Section on Histochem	LCS/NIMH
	T.A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.5

PROFESSIONAL:

.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent studies have identified a circadian pacemaker located within the suprachiasmatic nucleus (SCN) as a central component that regulates the rhythmic expression of a variety of mammalian circadian processes. The SCN performs three important functions. It entrains circadian rhythms to the daily light-dark cycle. It generates an endogenous circadian rhythm of approximately twenty-four hours which then controls the period of secondary processes. Finally, it integrates these rhythms into a single, functional circadian system with coordinated temporal relationships between multiple circadian rhythms. Many of these functions may be compromised in affective disorder.

There are two major visual inputs to the SCN of the hypothalamus. The first is a direct projection from the retina, the retinohypothalamic tract (RHT). The neurotransmitter of this projection has not been clearly identified, but its function is to facilitate entrainment to the environmental light-dark cycle. The second is an indirect visual projection from the intergeniculate leaflet (IGL) cells of the lateral geniculate nucleus (GHT) to the SCN. This projection utilizes neuropeptide Y (NPY) and appears to convey information specifically pertaining to the level of ambient illumination to the SCN. Since a) the GHT is one of only three major projections to the SCN, b) NPY is contained within the GHT and is the only transmitter identified that relays visual information to the SCN, c) the functional significance of the GHT and NPY is closely related to the circadian system, and d) decreased levels of NPY have been observed in affective disorder, we have begun to investigate the circadian and environmental regulation of NPY mRNA synthesis within the GHT.

Using in situ hybridization histochemical techniques, other investigators have recently demonstrated the presence of mRNA encoding for NPY in rat arcuate nuclei and cortex. Using in situ hybridization, we have identified NPY mRNA in the vicinity of the IGL cells. These results indicate that in situ hybridization may be a viable technique for further exploring a) the circadian regulation of NPY mRNA synthesis and b) the effect of different ambient light levels on the control of NPY mRNA. These experiments are currently in progress.

Project Description:

NPY is a thirty-six amino acid peptide present within a major visual projection to the mammalian circadian pacemaker. Evidence suggests that this neuropeptide conveys information pertaining to the intensity of ambient illumination to the circadian pacemaker. Our goal is to determine a) the circadian profile of NPY mRNA synthesis within the IGL cells of the lateral geniculate nucleus and b) the relationship between different levels of ambient illumination and levels of NPY mRNA encoded within the IGL.

Methods:

A 28 base pair oligonucleotide encoding for NPY amino acid sequence 1-28 was synthesized by OCS labs. The probe was labeled with either ^{32}P or ^{35}S using terminal deoxytransferase and purified by selective hydrophobic chromatography using NENSORP 20 columns (Dupont). Hamsters were decapitated during the middle of the light cycle and brains removed, frozen, and 12μ sections obtained throughout the extent of the IGL and adjacent sections and mounted onto slides. Tissue was fixed in formalin, dehydrated, delipidated and treated with various agents to reduce background staining. The labeled probe was applied to the section (500,000 cpm/section) and allowed to hybridize overnight. The tissue was then washed and dehydrated and exposed to either X-ray film for detection, or dipped in liquid film emulsion for higher resolution. Film and emulsion coated slides were allowed to expose for 1-6 weeks and then developed with Kodak D19 and rapid fixer.

Findings to Date:

Preliminary results demonstrate regional localization of NPY mRNA in the vicinity of the IGL of the lateral geniculate nucleus in animals evaluated during the light phase of the light-dark cycle. NPY mRNA was also identified within areas previously identified as encoding NPY mRNA such as the arcuate nucleus and cortex.

Significance to Biomedical Research:

Visual input to the mammalian hypothalamus is not completely understood. Some clinical evidence indicates high intensity visual input to the hypothalamus produces antidepressant effects in humans. The transmitters of visual information to the mammalian circadian pacemaker located in the suprachiasmatic nucleus have not been clearly identified. One possible transmitter is NPY contained within the projection from the thalamus to the hypothalamus; this projection may convey information about the level of ambient illumination to the hypothalamus. This project may identify a relationship between NPY and light intensity. Since bright light is reported to have antidepressant effects in humans, it is possible this research will identify a physiological link between bright light therapy and the circadian pacemaker of the hypothalamus.

Proposed Course:

These preliminary results indicate *in situ* hybridization is a viable technique for exploring the circadian profile of NPY mRNA within the IGL cells of the LGN. Our initial plans are to quantify levels of NPY mRNA within the IGL during the light and dark phases of the circadian cycle using densitometer image analysis. Second a dose-response experiment will be performed to assess the effect of light intensity on NPY mRNA levels. Third, if a circadian profile is identified during a light-dark cycle, the endogenous control of NPY mRNA will be assessed in appropriate constant lighting conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02430-01 CP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Effects of Antidepressant Drugs on Sensitivity of the Circadian Pacemaker to Light

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. C. Duncan Research Psychologist CPB/NIMH
Others: T.A. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH

COOPERATING UNITS (if any)

P.G. Sokolove, Professor of Biological Sciences, University of Maryland

LAB/BRANCH
Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: .5	PROFESSIONAL: .25	OTHER: .25
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CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

One of the functions of the mammalian circadian pacemaker is to entrain a variety of circadian rhythms to the daily light-dark cycle. The circadian pacemaker has the capacity to advance or delay its phase in order to maintain proper timing with the geophysical day-night cycle. The phase-advance and phase-delay mechanisms exhibit independent profiles of responsiveness to the light-dark cycle. Phase-advances show maximum responsiveness to light at dawn and phase-delays show maximum responsiveness to light at dusk. This project explores the effect of antidepressant drugs on the sensitivity of the circadian pacemaker to light.

Phase disorders of the sleep-wake cycle such as phase-delay sleep syndrome, or sleep disturbances that accompany depression prior to, or during drug treatment may be caused by the inability of the circadian pacemaker to properly advance or delay its phase in relationship to the external light-dark cycle. Antidepressant drug treatments may alter the sensitivity of the circadian pacemaker to light and thus establish non-pathological phase relationships between the pacemaker and the environment.

We have previously observed that the monoamine oxidase inhibitor, clorgyline, alters the responsiveness of the circadian pacemaker to light. Further, the effect of these compounds on threshold or saturation response was not been described. We have determined that chronic treatment with the monoamine oxidase inhibitor clorgyline increases the response threshold of the circadian pacemaker to light.

Project Description:

Disturbances of the sleep-wake cycle may be caused by a circadian pacemaker that lacks the capacity to properly entrain to the light-dark cycle. This dysfunction may result from a change in the sensitivity of the pacemaker to light. Previous data suggests that clorgyline, an antidepressant compound which produces increased daytime sleepiness in humans, alters the response of the circadian pacemaker to light. We are exploring the effect of clorgyline and other antidepressant drugs on the sensitivity of the circadian pacemaker to light.

Methods:

1) Experimental equipment

Measurements of wheel-running behavior of Syrian hamsters were collected on a laboratory computer as described in project report Z01 MH 02294-01 CP. In addition we have developed a controlled light chamber that delivers controlled quanta of light to hamsters that have been previously housed in continuous darkness. The controlled light chamber consists of three sections; a ventilated light source which utilizes a Sylvania FHS 300 W 82V tungsten halogen lamp and is controlled by a manual shutter, a collimator which controls the intensity and wavelength of the light and consists of an infrared filter, a neutral density filter and a narrow band filter (500 nm, 10 nm HB), and four individual compartments that temporarily house the test animals while light is administered.

2) The effects of chronic clorgyline on light sensitivity of the circadian pacemaker

Group housed Syrian hamsters are treated for two months with clorgyline (2 mg per kg per day) or saline using subcutaneously implanted mini-osmotic pumps. During drug treatment hamsters are transferred to individual containers containing running wheels. Hamsters were maintained in LD 14.5 : 9.5 for about one week and then maintained in continuous darkness for the remainder of the study (about three weeks) except for a single five minute pulse of light administered to hamsters on day eight of continuous darkness. The magnitude of the phase-shift response at each light intensity is determined by calculating the difference between the average time of wheel-running activity onset during the seven days before, and during the fourteen days after the light pulse.

Four light intensities with 6-8 animals per intensity have been tested for the two groups: .0137, .137, 1.37 and 13.7 $\mu\text{W} / \text{cm}^2$. Light is administered at two circadian phases: 1.5 and 6 hours after activity onset (CT 13.5 and CT 18 respectively). The sensitivity of the phase-shift response is determined as the quanta of light required to yield a threshold response, a half-maximum response and a saturation response.

3) Findings to Date:

Using a light intensity required to produce a half-maximum phase-shift response in normal animals, clorgyline diminished the magnitude of the response at CT 13.5 by 50% and diminished the response at CT 18 by 100%. When measured at CT 18, chronic clorgyline treatment increased the response threshold by approximately two orders of magnitude as compared to saline treated controls.

Significance to Biomedical Research:

A circadian pacemaker located in the mammalian hypothalamus controls the proper timing of behavior and physiology with the environment, and the proper timing of endogenous rhythms with each other. Because the magnitude of the phase-shift response varies as a function of light intensity, the level of ambient lighting can alter the phase relationship between the hypothalamus and the environment. The effects of antidepressant drugs appear to alter the sensitivity of the pacemaker to light. A formal description of these properties will provide information useful in improving treatment outcomes by properly matching drug-light interactions with a phase disorder

syndrome. In addition, these studies may provide insight regarding the source of the disorganized sleep-wake cycle that follow the chronic use of many antidepressant compounds.

Proposed Course:

We will completely explore the relationship between chronic clorgyline treatment and light sensitivity in Syrian hamsters. The light intensity-phase shift response relationship will be determined for both phase delays (CT 13.5) and phase advances (CT 18). Based on the results of this experiment, a drug representative of tricyclic antidepressant compounds may be evaluated to determine the class specific properties of these chemicals on pacemaker sensitivity.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00274-14 LCS

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Methods of Ionization in Mass Spectrometry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH

Others: Jeffrey P. Honovich, NRC Research Associate, SAB, LCS, NIMH

Tao-Chin Wang, Fogarty Fellow, LCS, NIMH

COOPERATING UNITS (if any)

Department of Pharmacology, George Washington University, Washington, DC

Oak Ridge National Laboratory, Analytical Chemistry Division, Oak Ridge, TN

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.75

PROFESSIONAL:

2.75

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The motion of ions in a dual cell Fourier transform ion cyclotron resonance spectrometer has been empirically measured and mathematically modeled in order to account for the analytical characteristics of this instrument. It is apparent that ions with significant kinetic energy cannot be successfully transferred between cells, and that additional focussing potentials are required to efficiently transfer laser desorbed ions.

A continuous flow-fast atom bombardment inlet and ionization system on a tandem quadrupole mass spectrometer is being tested for repetitive analyses of polar organic compounds in biofluids. Preliminary data has been obtained for several neurotoxins and biogenic amine metabolites which indicate a minimum detectability of 1-10 ng/sample. The specificity and accuracy of this analytical instrument is presently being evaluated.

Other Professional Personnel Engaged on Project:

Fred P. Abramson	Guest Worker	Professor, Department of Pharmacology, G.W. Univ. Washington, DC
Peter J. Todd	Collaborator	Research Scientist, Oak Ridge Nat'l Lab., Oak Ridge, TN

Project DescriptionObjective:

Improvement in the specificity and detectability of complex organic compounds in biological matrices requires new developments in mass spectrometric instrumentation. Surface ionization techniques (laser desorption, fast atom bombardment) are being explored for the trace analysis of neuropeptides, neurohormones, and drug metabolites. Fourier transform ion cyclotron resonance spectroscopy (FT-ICR) is being explored as a high sensitivity and high performance mass analyzer. Continuous flow-fast atom bombardment is being tested for quantitative analysis of polar organic metabolites. The objective of these studies is to develop and utilize analytical procedures to resolve problems which cannot be solved with conventional gas phase instrumentation.

Methods Employed:

Mass spectrometric instrumentation is designed, built, modified, or purchased as required to meet the above objectives.

Major Findings:

During the current year, the motion of ions in a dual cell Fourier transform ion cyclotron resonance (FT-ICR) spectrometer was measured and successfully mathematically modeled using electron impact generated ions. In the dual cell FT-ICR, the cells are separated by a common trapping plate with a 2 mm hole known as the conductance limit, which is a means of providing two independently pumped regions for ion creation and detection. This design has been promoted for the laser desorption ionization of neuropeptides and other biomolecules, followed by their transfer to a low pressure analyzer cell and high sensitivity detection. Failure of the dual cell concept to work as anticipated prompted the present modeling studies. Ion motions which depend on the z-axis electric fields (trapping motion) and those in the x-y plane (magnetron motion) were observed.

To study the trapping oscillation, a collection of ions was first produced in the source cell. The conductance limit was then grounded to allow the ions to shuffle back and forth between cells until transfer was stopped by raising the conductance limit potential to that of the trapping plates. By analyzing the ion population of each cell vs the time the conductance limit was electrically open, information on both ion transfer efficiency and the potential energy surface inside the cell was obtained. We have determined the non-harmonic nature of our cell configuration during the transfer process by comparing the experimental curves with a computer model. These results are in qualitative agreement with numerical calculations of the cell potentials.

In another set of experiments, ions were produced and trapped on the source side for various periods of time before allowing transfer to the analyzer cell for detection. In this way we have observed oscillatory variations in the transfer efficiencies where the ions move off alignment from the 2 mm hole in the conductance limit. By studying the mass, trap voltage, ion density, and pressure dependencies of this effect, we ascribe it to magnetron motion involving the X-Y components of the electric field in the cubic ICR cell. In addition to providing information on ion motion in an ICR cell, the results have direct applications to ion transfer procedures for the use of the dual-cell as an analytical and research tool.

A new triple stage quadrupole mass spectrometer has been configured with a prototype continuous flow-fast atom bombardment (CF-FAB) inlet/ionization system and is being tested and refined for rapid and repetitive quantitative analysis of polar organic compounds. The discriminatory power of tandem mass spectrometry will be used to differentiate between compounds of the same nominal mass but yielding different fragment ions. Problems of high fluctuating chemical background and beam instability have been reduced substantially with CF-FAB because the sample flows onto the probe tip for a defined time period (approx. 30 sec), thus permitting conventional chromatographic data handling procedures to be applied. Glycerol-water solutions of the compounds being analyzed are injected through a micro-bore liquid chromatographic inlet system which maintains a constant flow of a thin film on the probe surface. A xenon FAB source and a cesium ion gun have been tested as primary ion sources. The cesium ion gun functions without increasing the pressure in the ion source region and thus offers some advantage for tandem mass analysis during which collision gas otherwise adds a significant pressure burden to the mass analyzer.

Preliminary quantitative studies have been conducted with the following compounds with their respective stable isotope labelled internal standards: 1-methyl-4-phenylpyridine (MPP⁺); 6-hydroxymelatonin sulfate, tryptophan, and β -N-methylamino-L-alanine (BMAA). All have exhibited good detectability with levels as low as 10-50 ng/sample. Greatest effort has been directed toward 3-methoxy-4-hydroxyphenylethyleneglycol-sulfate (MHPG-SO₃⁻ K⁺). However, a deuterated internal standard has not as yet been prepared in sufficient quantity to allow MHPG-SO₃⁻ quantification in human urine. The CF-FAB system will be developed for accurate, benchmark determinations of metabolites measured by less direct methodology to reduce significant inter-laboratory variability in metabolite analyses.

The organic ion imaging microprobe analyzer project in Dr. Todd's laboratory at Oak Ridge National Laboratory has been tested with solutions of MPP⁺, and its imaging capabilities demonstrated. However, to improve the lenses and ion transmission, the instrument is being re-configured. Dr. Todd has applied an ion trajectory calculation modeling program to determine lens designs for the cesium ion gun used for CF-FAB at NIH in collaboration with Dr. Wang and has initiated simulations of ion trajectories following laser desorption in the NIH FT/ICR in collaboration with Dr. Honovich.

The microwave discharge interface project in collaboration with Dr. Abramson at George Washington University is being applied to the analysis of ¹⁵N and ²H labelled variants of BMAA synthesized by Dr. M. Duncan (Z01 MH 00279-06 LCS).

Significance to Biomedical Research:

Structure elucidation of unknown compounds in complex mixtures, or the specific detection and quantification of known compounds and their isotopic variants remain important areas of biomedical research. Polar, non-volatile compounds are frequently encountered in neurochemistry, and the ionization methods and instrumentation being tested are particularly relevant to the analysis of neuropeptides and neurohormones.

Proposed Course:

An extraction and focussing lens system will be designed for the FT/ICR spectrometer in order to make it useful for samples of biomedical interest which are limited in quantity.

A focussed cesium primary ion gun will be built and tested for CF-FAB analyses. CF-FAB analyses of $\text{MHPG-SO}_3^-\text{K}^+$ will be refined for maximum sensitivity, specificity, and accuracy using micro-bore columns and/or tandem mass spectrometric techniques.

Publications:

Honovich JP, Markey SP. Studies of ion motion in an ICR cell by dual cell FTMS. Proceedings of the 36th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics, June 5-10, 1988.

Markey SP. Principles and applications of mass spectrometry in clinical chemistry. Clinical Chemistry - Proceedings of the XIII International Congress of Clinical Chemistry & the VII European Congress of Clinical Chemistry, The Netherlands, June 28-July 3, 1987.

Markey SP, Wang T-CL, Shih M, Boni R, Duncan M, Yang S-C. Quantitative analysis of polar compounds by continuous flow FAB. Proceedings of the 36th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics, June 5-10, 1988.

Wang TC, Markey SP. Effects of Apodization and 12-bit ADC on intensities in FT/ICR. The Ohio State University FT/MS Newsletter 1987;8:29.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00277-09 LCS

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis of Stable Isotope-Labeled Compounds

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH

Others: Mark Duncan, Fogarty Visiting Fellow, CNB, NINCDS

COOPERATING UNITS (if any)

Office of the Director, IRP, NINCDS

Laboratory of Chemical Physics, NIDDK

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Stable- and some radioisotope-labeled compounds have been synthesized to support other laboratory projects. Structural analogues of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have been prepared for animal testing. ²H and ¹⁵N isotopomers of β-N-methylaminoalanine have been synthesized. Methods suitable for the selective sulfation of phenols in the presence of alcohols are under study.

Objectives:

The synthesis of labeled compounds is an integral program component in the investigation of metabolism and distribution of endogenous and xenobiotic compounds. Other projects in the SAB require labeled or structural analogues in order to trace metabolic pathways, determine kinetics, or accurately measure trace quantities with an internal mass standard.

Methods Employed:

Conventional routes of organic chemical synthesis employing isotopes and general chemicals have been used. Quantities (5 g) of 1-butyl-4-phenylpyridine (BPP⁺) were prepared sufficient for use as both an internal standard for HPLC assays and for animal and cell culture toxicity testing (Z01 MH 00279-06 LCS). A portion of the BPP⁺ was reduced with sodium borohydride to prepare 0.5 g BPTP HCl (1-butyl-4-phenyl-1,2,3,6-tetrahydropyridine). Small quantities (0.1 g) of 1-methyl-4-(4'-methylamino)phenyl-1,2,3,6-tetrahydropyridine) were prepared for animal testing from previously synthesized 4'-amino MPTP.

²H- and ¹⁵N isotopomers of the putative neurotoxin β-N-methylaminoalanine (BMAA) were prepared by modification of published syntheses and then rigorously characterized to confirm both structure and purity. This method involves a Michael-type condensation between 2-acetamidoacrylic acid and methylamine, to form the intermediate N-acetyl BMAA, which was hydrolyzed to furnish the amine hydrochloride. By carrying out this reaction on a 5 gram scale we have been able to prepare sufficient material to act as a reference compound and to employ in animal testing. Thorough characterization was essential because the literature value for the melting point of the hydrochloride salt was previously reported as 165-167°C, but our repeated synthesis and melting point determinations gave a value of 181-182°C. GC/MS of a variety of N-fluoroacyl esters was performed in both the EI and NCI modes.

Substitution of ²H₃-methylamine for methylamine, and refinement of the procedure to improve the yield of product, allowed preparation of 65 mgs of the intermediate N-acetamyl compound. This material was characterized by NMR and then converted to free-²H₃-BMAA by acid-catalyzed hydrolysis. The acidic aqueous solution was diluted and used directly as an internal standard without further purification. Gas chromatography-mass spectrometry of the pentafluoropropionyl (PFP) and trifluoroethanol (TFE) derivative of the synthetic product was used to establish the chemical and isotopic purity. GC analysis of the derivatized product was shown to consist of only one component. The base peak in the EI spectrum of a scan from this GC peak was shown to be increased by 3 mass units (i.e., from m/z 190 to 193) when compared with authentic BMAA.

In collaboration with Drs. Herman Ziffer and Yulin Hu (NIDDK) we have also undertaken the synthesis of [¹⁵N]-BMAA for use in animal studies aimed at establishing the catabolic fate of administered BMAA. For this synthesis it was necessary to optimize the yield for the reaction between the acrylic acid and methylamine by incorporating a recycling step. We have now been successful in preparing 400 mg of pure [¹⁵N]-L-BMAA from 2 g of labelled methylamine hydrochloride.

²H-labelled standards of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate and 6-hydroxymelatonin sulfate are required for quantitative mass spectrometric analyses. Several sulfation methods are under investigation to provide crystalline standards rather than the enzymatically produced trace amounts previously available. New isolation methods have been devised and tested for separating both synthetic product sulfates and urinary conjugates from complex mixtures using ion pairing reagents and reverse phase resins.

Major Findings:

The use of each of these synthetic products is described in other annual reports (Z01 MH 00279-06 LCS, Z01 MH 02384-02 LCS).

Significance to Biomedical Research:

The availability of labeled compounds is frequently the limiting step in metabolism projects. A program in analytical biochemistry requires continuing synthetic efforts to prepare stable and radioisotope analogues for the timely and efficient solution to metabolism projects.

Proposed Course:

Synthesis of labelled analogues of BMAA and related neurotoxins will continue. Methods of sulfation suitable for the syntheses of ²H labelled MHPG and 6-hydroxymelatonin sulfates will be refined.

Publications:

Weisz A, Markey SP. Synthesis of D/L-norepinephrine-(phenyl-U-¹³C), J Lab Compounds & Radiopharm 1988;25:103.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00279-06 LCS

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology of Neurotoxins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Sanford P. Markey, Chief, Section on Analytical Biochemistry

Others: Jan Johannessen, Senior Staff Fellow, SAB, LCS, NIMH

Song-cheng Yang, Visiting Scientist, SAB, LCS, NIMH

Hiroya Ikeda, Visiting Worker, SAB, LCS, NIMH

Mark Duncan, Visiting Fellow, IRP, NINCDS

Miles Herkenham, Senior Investigator, LNP, NIMH

Kris Bankiewicz, Visiting Fellow, SNB, NINCDS

Ann Marini, Senior Staff Fellow, CNB, NINCDS

Thomas J. Sobotka, Senior Investigator, Division Toxicology, CFSAN, FDA

COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NIMH

Clinical Neuroscience Branch

CFSAN, Food and Drug Administration

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.05

PROFESSIONAL:

3.75

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The metabolism and mechanism of action of the parkinsonian syndrome inducing neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and its amine analogue 4'-amino MPTP have been studied in mouse, dog, and monkey. The identification of MPTP metabolites in mouse brain, the concentrations of the principle metabolite 1-methyl-4-phenylpyridine (MPP+) in mouse and monkey brain, and the relationship between neurotoxicity and MPP+ concentration supports the causal relationship between MPP+ accumulation in target brain cells and neural degeneration. Structurally related environmental neurotoxins are being sought using an immunoassay to MPP+; functionally related neurotoxins are being sought with a cultured cell system. Low doses of MPTP produce long lasting (6 week) pharmacological effects in dogs suggesting that related neurotoxins might be detectable in exposed populations. The testing of 4'-amino MPTP in dogs has been completed, and it has been shown to be a less specific neurotoxin than MPTP, perhaps producing a better animal model of the human disease.

A gas chromatography-mass spectrometric assay of plant extracts containing the putative neurotoxic amino acid β -N-methylaminoalanine (BMAA) has been developed. The assay is being applied to cycad seeds from Guam in order to test the hypothesis that BMAA causes the high incidence of amotrophic lateral sclerosis/Parkinsonism dementia among native Guamanians.

Objectives:

The neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in man and monkey result in a parkinsonian syndrome which is nearly indistinguishable from idiopathic Parkinson's disease. By determining the mechanism of MPTP's action in destroying a specific sub-set of dopamine-rich cells in primate brain, rationale therapy to slow or prevent the progressive idiopathic disease process in man might be designed. Further, environmental toxins which may have mechanisms similar to that of MPTP are being investigated. The metabolism of a compound implicated in the high incidence of a parkinsonian syndrome on Guam, α -amino- β -methylaminopropionic acid (BMAA) is being studied to determine if there are characteristic urinary metabolites to indicate dietary exposure.

Methods Employed:

MPTP toxicity is being studied by: qualitative and quantitative observation of animal behavior and locomotion; neurochemical determination of catecholamines and their metabolites in specific brain regions by high pressure liquid chromatography with electrochemical detection (HPLC-EC); determination of the pattern of MPTP distribution, metabolism, and excretion, using radio and stable-labelled MPTP (^3H , ^{14}C , ^2H) in mouse and monkey; autoradiography of tissue exposed to labelled-MPTP (Z01 MH 01090-11); and mass spectrometry of isolated metabolites. Enzyme-linked immunoassay and radioimmunoassay procedures are being used to detect MPTP, MPP⁺, and structurally related compounds.

Survival of rat cultured cerebellar granule cells is being used as a functional measure of neurotoxicity in the search for neurotoxins related to MPP⁺.

A combination of mass spectrometric methods (GC-MS, continuous flow-fast atom bombardment MS, and microwave discharge interface MS) are being used to determine both the occurrence of BMAA in plant material and its metabolism in animals.

Major Findings:

Studies on the metabolism of $^{14}\text{C}_6$ -MPTP in mice and monkeys revealed that the distribution and concentration of MPP⁺ in monkey brain is consistent with it being the operative neurotoxin in that concentrations of 1-100 μM have been measured in monkey brain, and similar MPP⁺ concentrations are toxic to dopaminergic cells in culture. Other MPTP metabolites identified in mouse brain (demethylated MPTP, 1-methyl-4-phenyl-2-pyridone) probably derive from liver metabolism and are not related to neurotoxicity. The inverse relationship between tissue bound metabolites and neurotoxicity and the direct relationship between MPP⁺ concentration and neurotoxicity support the causal relationship between MPP⁺ and neural degeneration.

The issue of environmental neurotoxins as being causative factors in idiopathic Parkinson's disease has prompted both immunologic and toxicological studies. We have progressed in the immunoassay of substances in Parkinsonian brain which may be related to MPP⁺ to a quantitative ELISA assay, refined with the inclusion of an enzyme amplification step to permit detection of MPP⁺ in

the concentration range of .5 to 1000 nM. This ELISA assay has been tested with compounds closely related to MPP⁺ and those which might be present in biological extracts to determine cross-reactivity values.

An extraction procedure for brain tissue has been devised and tested which solubilizes MPP⁺ and related compounds in perchloric acid; removes amines by extraction into organic solvents; and concentrates quaternary amines related to MPP⁺ on ion exchange resins which can be eluted quantitatively in characteristic fractions. Extracts of both normal and Parkinson disease brain tissues have not, to date, shown any MPP⁺ immunoreactivity in fractions processed as described.

In an effort to examine the possibility that environmental neurotoxins might be responsible for degeneration of nigrostriatal neurons, we have undertaken a two stage study in the dog in collaboration with Dr. Tom Sobotka of the FDA. We have first sought to define a dose-response relationship for MPTP for the dual purpose of gauging the sensitivity of the nigrostriatal system to a single dose MPTP and to see if prolonged neurochemical changes can result from subtoxic doses (doses which do not lead to loss of nigral neurons) which might be indicative of the persistence of the MPTP metabolite MPP⁺ in the brain. Beagle dogs, an MPTP sensitive species, were dosed once with 2.5 mg/kg (the standard toxic dose which produces loss of virtually all of the SNc cells), 1.0 mg/kg, 0.5 mg/kg, or 0.1 mg/kg. Open field behavior was monitored and the animals were sacrificed 6 weeks after injection. Neurochemical analyses of the striata indicate that dopamine levels do not fall significantly until the 1.0 mg/kg dose, and then only by 20%, and no loss of cells was detected. The 2.5 mg/kg dose results in the complete loss of striatal dopamine and complete cell loss in the SNc. In contrast the dopamine metabolites DOPAC and HVA show a dose related decline starting at 0.1 mg/kg. The impressive fact that 1/25th of the toxic dose results in a decrease in DOPAC 6 weeks after a single injection suggests that MPP⁺, which we showed previously persists in nigrostriatal dopamine terminals for long periods is exerting a pharmacological effect, possibly an inhibition of presynaptic MAO-A. This possibility is now being considered by measuring levels of MPP⁺ in these brains and seeing if they relate to the loss of DOPAC and HVA and also using in vitro techniques to investigate the ability of low concentrations of MPP⁺ to inhibit selectively presynaptic pools of MAO-A.

Because extremely low doses of MPTP cause prolonged neurochemical changes, we have begun to examine the effects of repeated low doses of MPTP, a situation likely to mimic repeated exposure to an environmental toxin. Dogs were treated with 0.2 mg/kg MPTP at 1 week intervals for either 5 weeks or 12 weeks (cumulative dose 1.0 or 2.5 mg/kg). There were no significant behavioral changes. Brain tissue and CSF will be analyzed for regional catecholamine content and neuropathologic examination of the brains will be undertaken.

The persistent changes in metabolites of DA centrally after low doses of MPTP prompted examination of the effects of toxic doses of MPTP on peripheral catecholamine metabolites. As a part of a toxicity evaluation of 4'-amino MPTP in the dog, weekly plasma samples were taken and plasma catecholamines were measured in collaboration with Dr. David Goldstein of NHLBI. As with the CNS, MPTP caused a profound loss of catecholamine metabolites which depend on the action of MAO, DHPG and DOPAC. The loss of DHPG is approximately 90% one week after treatment and this decrease persists for at least 6 weeks (the end

of the experiment). Thus we have demonstrated that plasma catecholamine metabolite levels can be used as a sensitive (albeit non-selective) marker for the exposure to toxic amounts of MPTP and presumably other MPTP-like toxins, if they exist.

The evaluation of the behavioral, biochemical, and neuropathologic effects of 4'-amino MPTP in the dog are now nearing completion. 4'-amino MPTP causes a profound loss of striatal dopamine (> 99%) and a virtual loss of cells within the substantia nigra pars compacta (SNc). Considerable cell loss has also been noted in the locus coeruleus and the ventral tegmental area, a departure from the narrow specificity of MPTP. Additional regional measurements of catecholamines are now underway to determine the regional extent of the loss. The neurotoxic effects (cell loss) are prevented by pargyline, suggesting that this neurotoxin acts in a similar manner to MPTP and, in fact, in vitro experiments have detected the generation of 4'-amino MPP⁺. Behaviorally, 4'-amino MPTP is similar to MPTP, except that the effects are more pronounced and longer lasting. There is extreme hypokinesia which lasts for 2-3 weeks and is accompanied by pupillary dilation, lack of a pupillary response to light, postural tremor and, after recovery of motor function, freezing episodes. While the dog is a poor behavioral model for parkinsonism, the neurochemical and neuropathologic changes are the same as in the monkey and these results, indicating that 4'-amino MPTP involves the VTA and LC, suggest that this toxin may prove to produce a better model of human parkinsonism than MPTP.

For over 30 years the unusually high incidence of amyotrophic lateral sclerosis and associated parkinsonism dementia (ALS-PD) among the Chamorro people native to the Mariana Islands has been linked to the consumption of the seeds of *Cycad circinalis* L., or false sago palm. However, despite an intensive research effort, it has been difficult to find experimental evidence to support this widely held belief. Recently, evidence was presented that 2-amino-3-(methylamino)-propionic acid (synonyms, β -methylamino-L-alanine or BMAA), an excitatory neurotoxin known to be present in the seeds of *Cycad circinalis* L., is responsible for the well-characterized neuronal degeneration characteristic of this disease. However, the presence of BMAA in the foods prepared and consumed by the Chamorros has not been established and the putative relationship between BMAA in *Cycad circinalis* L. and Guamanian ALS-PD requires more rigorous testing.

To investigate this hypothesis we needed to be able to quantify BMAA in a range of sample types (including food samples) so that we could establish a link between BMAA and Guamanian ALS-PD. We have developed a gas chromatography-mass spectrometry (GC/MS) method using a deuterium isotopomer (Z01 MH 00277-09 LCS) as internal standard to ensure precise, low-level quantification. The assay we have developed has the versatility to allow BMAA quantification in a wide variety of sample types. Using GC/MS in the electron impact mode (EI) the PFP and TFE derivative of BMAA gives an intense ion at m/z 190 which is ideally suited for quantification. Additionally, the m/z 465 ion can be used for confirmation. Capillary gas chromatography has been used throughout. The standard curve indicated a limit of detection of approximately 5 picogram of BMAA injected. The precision of the assay in the low picogram range has been shown to be excellent.

Significance to Biomedical Research:

The MPTP-lesioned primate has been firmly established as a useful animal model of idiopathic Parkinson's disease in man. The mechanism of action of MPTP may be relevant to the human disease process, and attempts to identify neurotoxic environmental agents may lead to preventative measures for a very common disease of aging.

Proposed Course:

The screening of human brain extracts for neurotoxic materials will proceed using a functional assay of the effects of extracts on cultured cells. Additionally, adrenal tissue which stores MPP⁺-like substances will be tested both for immunoreactive and functional neurotoxins. The quantitative assay of BMAA and related neurotoxic materials in Cycad extracts will be pursued.

The theory that MPTP-like neurotoxins play a role in the etiology of some cases of parkinsonism will be addressed. To this end we have established a collaboration with Dr. Tom Hutton at Texas Tech University. Dr. Hutton has been struck by the high incidence of parkinsonism amongst younger agricultural workers in and around Lubbock Texas and has been collecting plasma samples from three groups; controls, young parkinsonian patients, and agricultural workers who are asymptomatic, but who are currently working in areas of high pesticide use (cotton mills). We will analyze the samples for catecholamine metabolites in the near future. If agricultural workers who are asymptomatic are being exposed to toxic doses of an MPTP-like neurotoxin, we might expect to see some changes in the CA metabolites.

Visualization of 4'-amino MPTP and its metabolite 4'-amino MPP⁺ in brain tissue by immunohistochemical methods will begin. Localizing the distribution of the toxin and its metabolites at the cellular and ultrastructural level will help determine its mode of action.

Publications:

Kopin IJ, Markey SP. MPTP toxicity: Implications for research in Parkinson's disease. *Ann Rev Neurosci*, 1988;11:81-96.

Markey SP, Ikeda H, Yang S-C, Markey CJ, Marini AM, Johannessen JN. Search for environmental or endogenous neurotoxins related to MPTP. In: Hefti F, Weiner WJ, eds. *Progress in Parkinson Research*. Plenum, New York, 1988, in press.

Markey SP, Yang S-C, Johannessen JN, Burns RS, Herkenham M, Bankiewicz K. Mechanisms of MPTP toxicity. *Proceedings of the Catecholamine Symposium*, Israel, 1987.

Yang S-C, Johannessen JN, Markey SP. Metabolism of ¹⁴C-MPTP in mouse and monkey implicates MPP⁺, and not bound metabolites, as the operative neurotoxin. *Chemical Research in Toxicology*, 1988, in press.

Yang S-C, Markey SP, Bankiewicz KS, London WT, Lunn G. Recommended safe practices for using the neurotoxin MPTP in animal experiments. *Lab Animal Sci*, 1988, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02384-02 LCS

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Quinolinic Acid Metabolism: Role in Neuronathology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Melvyn P. Heyes, Visiting Associate, LNP, NIMH

Others: Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH

Riccardo L. Boni, Fogarty Visiting Fellow, LCS, NIMH

COOPERATING UNITS (if any)

Laboratory of Neurophysiology

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

L-tryptophan (L-TRP) is an essential amino acid that is metabolized in the brain to serotonin (5-HT) and through the kynurenine pathway to quinolinic acid (QUIN). Studies in experimental animals have established QUIN as a potent neurotoxin and convulsant and potentially QUIN may have a role in human neurodegenerative and convulsant disorders. Furthermore, the rate of metabolism of L-TRP through the kynurenine pathway may influence L-TRP concentrations in brain and therefore the availability of L-TRP for conversion to 5-HT. To investigate the role of QUIN in human neuropathology and study L-TRP metabolism through the kynurenine pathway, we have perfected methods to quantify QUIN, 3-hydroxykynurenine (3-HKYN) and L-TRP in brain. Completed studies to date demonstrate that during systemic L-TRP loads, changes in the concentrations of QUIN and 3-HKYN far exceed the magnitude of changes in 5-HT and 5-HIAA. Severe insulin-induced hypoglycemia increases brain QUIN and it remains to be determined whether QUIN contributes to neuropathology associated with hypoglycemia. 4-chloro-3-hydroxyanthranilic acid inhibited brain 3-hydroxyanthranilic acid both in vivo and in vitro.

Objective:

The mechanism responsible for causing nerve cell death in human neurodegenerative disorders are unknown, but it has been proposed that over-production of amino acid agonists of excitatory amino acid receptors may be responsible because activation of these receptors leads to nerve cell death in experimental animals. Quinolinic acid (QUIN), is such a neurotoxin and is also a convulsant. QUIN is synthesized from L-TRP via metabolism through the kynurenine pathway, but very few studies have investigated this pathway in brain. The kynurenine pathway may be particularly important in the regulation of brain L-TRP concentrations and therefore the rate of synthesis of the neurotransmitter, 5-HT.

The objective of the present project have been to develop a method to quantify QUIN in brain and CSF, quantify QUIN synthesis under situations of increased L-TRP, develop a method to quantify 3-hydroxykynurenine (3-HKYN), another potential convulsant and begin measures of QUIN concentrations in human neuropathologic disorders.

Methods:

[¹⁸O]-QUIN was prepared by heating QUIN in [¹⁸O]-water/3 N HCL for 48 hours at 80°C. A method was perfected for purification of brain, CSF, and plasma perchloric acid extracts where samples are washed with chloroform and QUIN isolated by ion exchange chromatography. Samples are derivatized to the dihexafluoroisopropanol ester without exchange of [¹⁸O]-QUIN by heating samples with hexafluoroisopropanol (HFIP) with trifluoroacetylimidazole as catalyst. Samples are then analyzed using a modified Finnigan 3200 negative chemical ionization mass spectrometer connected to a newly incorporated capillary column gas chromatograph. Samples of CSF are collected from human volunteers at the NIH and other institutions. Postmortem human brain samples are usually received as perchloric acid extracts, brain aliquots or whole brains. Animals are either male Sprague Dawley rats or C57Bl/6NCR mice. The assay now uses the same procedure for all samples. The QUIN assay has also been applied to measure QUIN in striatal dialysates in rats. These measures allow a quantitative measure of QUIN concentrations in the extracellular fluid and enable measures to be made in the same animal over time.

A method was developed for the automated quantification of L-tryptophan and deuterated L-tryptophan (2', 4', 5', 6', 7'-d₅). A number of derivatization schemes were evaluated. Conversion of tryptophan and deuterated tryptophan to their heptafluorobutyryl methyl esters gave the best derivatives for GC/MS analysis. Reaction conditions were optimized for maximum derivatization and minimum deuterium exchange. Derivatized standards were analyzed by GC/MS on a HP-MSD. Standard curves were linear over the range of concentrations examined, 20-20,000 pg/ L injected.

A solid phase extraction method (ion exchange resin) for the purification of tryptophan and deuterated tryptophan from biological matrices is presently being developed. This method will be optimized for maximum recovery of L-tryptophans (endogenous and stable-isotope labeled) and minimum interferences in subsequent GC/MS analysis.

A method to measure brain 3-HKYN was developed based on the HPLC separation of 3-HKYN in perchloric acid extracts and detection by electrochemical oxidation.

Major Findings:

Systemic L-TRP loads markedly increased QUIN concentrations in both blood and brain. In a collaborative study with Dr. M. During, Yale University, striatal dialysates showed that at a dose of 250 mg/kg L-TRP, QUIN concentrations increased over 200-fold, reaching a maximum value at about 2.5 hr after the systemic injection. In contrast, the concentrations of 5-HT and 5-HIAA were only increased up to 6-fold. This observation provides the first evidence that during period of increased brain L-TRP concentrations that flux through the kynurenine pathway may serve to regulate L-TRP concentrations in brain. It remains to be determined whether these marked increases in QUIN are neurotoxic.

In a collaborative study with Dr. Roland Auer, University of Calgary, Canada, we have studied the effects of insulin-induced hypoglycemia on regional brain QUIN content. Profound insulin-induced hypoglycemia is associated with neurodegeneration that is attenuated in severity by antagonists of NMDA receptors. Hypoglycemia increases L-tryptophan (L-TRP) concentrations in brain and could increase the concentration of quinolinic acid (QUIN), a neurotoxin, convulsant, and agonist of NMDA receptors. Therefore, we investigated the effects of 40 min of severe hypoglycemia and 1 hr of normoglycemic recovery on concentrations of QUIN, L-TRP, 5-HT, and 5-HIAA in frontal, parietal, and occipital cortices, striatum, thalamus, hippocampus, and cerebellum in male Wistar rats. In addition, dopamine, DOPAC, and HVA concentrations were also measured in striatum. QUIN concentrations increased 2- to 3-fold during hypoglycemia and increased 2- to 3-fold further during recovery in all regions examined. 5-HT decreased > 50% and 5-HIAA increased > 2-fold during hypoglycemia and recovery in all brain regions examined. In striatum, dopamine was depleted during hypoglycemia but returned to control values during recovery. Striatal DOPAC and HVA concentrations increased > 2-fold during hypoglycemia and recovery. We conclude that hypoglycemia increases brain QUIN synthesis and increases 5-HT release throughout the brain and dopamine release in striatum.

In liver 4-chloro-3-hydroxyanthranilic acid (Cl-HAA) inhibits 3-HAA/OX. To determine whether brain 3-HAA/OX is also inhibited by Cl-HAA, 3-hydroxyanthranilic acid (3-HAA) was injected into the cisterna magna of rats through chronic indwelling cannulae either with or without Cl-HAA. Regional brain QUIN quantitated by gas chromatography/mass spectrometry and [¹⁸O]-QUIN as internal standard. 3-OHAA markedly increased QUIN concentrations in all brain regions measured 10- to 83-fold. 4-Cl-3-OHAA attenuated these increases between 77% to 55%. These observations indicate that Cl-HAA is an inhibitor of brain 3-HAA/OX.

A collection of CSF and post mortem brain tissue has been collected from several centers in the USA and Canada. The concentrations of QUIN will be quantified.

Significance to Biomedical Research:

Neurodegenerative disorders are a major group of pathologic human diseases affecting all age groups and generally require costly and chronic management of limited success and minimal cure-rates. Most disorders are idiopathic. Increases in brain QUIN concentrations are a mechanism by which brain cells could die in these disorders and strategies to reduce brain QUIN concentrations or antagonize QUIN 'receptors' offer new approaches to therapy. Our efforts to develop a highly specific, sensitive and accurate assay for QUIN in tissue and body fluids and opens new directions in neurotoxicology.

Proposed Course:

Four linked approaches will be pursued over the coming year.

1) Cerebral Metabolism.

Continuation of studies in rodents on the metabolism of QUIN in brain. These will include the effects of diet, circulating amino acid concentrations and the effect of drugs and agents already known to influence L-TRP metabolism.

2) Animal Models of Human Neuropathology.

Both ischemia and hypoglycemia induce neurodegeneration which can be blocked by antagonists of NMDA-type excitatory amino acid receptors, suggesting that activation of these receptors mediate nerve cell damage in these situations. QUIN is a potent agonist of these receptors and mediates at least some of its neurotoxic effects by their activation. Perhaps QUIN is involved in the neuropathology of these conditions. This hypothesis is directly testable using the assay for QUIN we have developed. Studies of PYR will continue, particularly with respect to the effects of diet.

3) QUIN Concentrations in CSF.

Measurement of QUIN in the CSF in humans may open a window into QUIN metabolism in brain. If so, it may be possible to determine ongoing QUIN metabolism in human neuropathological states. To investigate how well QUIN concentrations in CSF reflect brain QUIN levels, rhesus monkeys will be given treatments which have been shown to either increase or decrease QUIN concentrations in brain of rodents, and the concentrations of QUIN in CSF will be determined. Later, postmortem studies will be done to fully characterize the relationship between QUIN in brain and CSF.

4) QUIN Concentrations in Human Neuropathology.

QUIN concentrations will be measured in samples of CSF and brain in patients suffering from and who have died from a variety of neurodegenerative, convulsant, and motor disorders, including AIDS. This result will be pursued in the next fiscal year.

Publications:

Heyes MP. Hypothesis: A role for quinolinic acid in the neuropathology of glutaric aciduria type I. Can J Neurol Sci 1987;441-443.

Heyles MP, Markey SP. (¹⁸O)Quinolinic acid: Its esterification without back exchange for use as internal standard in the quantification of brain and CSF quinolinic acid. Biomed Environ Mass Spectrom 1988;15:291-293.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00351-14 LCS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Pharmacology of the Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David C. Jimerson, M.D. Chief, Section on Biomedical Psychiatry LCS

COOPERATING UNITS (if any)

SAB, LCS, NIMH; SCBB, LCS, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Biomedical Psychiatry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Work on this projected has been discontinued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02289-04 LCS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychobiology of Eating Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David C. Jimerson, M.D. Chief, Section on Biomedical Psychiatry LCS

COOPERATING UNITS (if any)

SCN, LCS, NIMH; SCP, LCS, NIMH; SCN, BPB, NIMH; CNG, NIMH; SCS, NSB, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Biomedical Psychiatry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Work on this project has been incorporated into project Z01 MH 02432-01 CNE.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00332-10 LCS
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Animal Models for the Study of Neuropharmacologic Effects		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Charanjit S. Aulakh, Ph.D., Staff Fellow, Section on Clinical Neuropharmacology, LCS, NIMH		
COOPERATING UNITS (if any) Laboratory of Cerebral Metabolism, National Institute of Mental Health		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Clinical Neuropharmacology		
INSTITUTE AND LOCATION National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.6	PROFESSIONAL: 1.3	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Administration of the 5-HT_{1A} receptor agonist, <u>8-hydroxy-2(di-n-propylamino) tetralin (8-OHDPAT)</u>, to rats produced dose-dependent decreases in food intake and hypothermia, increases in plasma prolactin and corticosterone, and a decrease in plasma growth hormone. Pretreatment with the nonselective 5-HT receptor antagonist, metergoline, did not affect 8-OHDPAT-induced decreases in food intake but attenuated the prolactin release, and also potentiated 8-OHDPAT-induced hypothermia. Long-term (21 days) treatment with the <u>monoamine oxidase (MAO) type A</u> inhibiting antidepressant, <u>clorgyline</u>, but not the <u>tricyclic antidepressants</u>, <u>clomipramine</u> and <u>imipramine</u>, attenuated the hypothermic response to 8-OHDPAT. In another study, short-term (2-6 days) or long-term (21-25 days) treatment with clorgyline potentiated <u>fenfluramine</u>-induced suppression of food intake but did not affect fenfluramine-induced suppression of locomotor activity. On the other hand, long-term but not short-term imipramine treatment attenuated fenfluramine-induced decreases in food intake but not locomotor activity. These results indicate that various agents effective in different types of affective disorders exert differential modulatory influences on serotonergic mechanisms regulating food intake, locomotor activity, temperature, and neuroendocrine changes <i>in vivo</i>.</p> <p>In a separate series of studies, the <u>fawn-hooded (FH) rat strain</u> was found to be significantly less sensitive to the food intake suppressant effects of <u>m-chlorophenylpiperazine</u> (m-CPP, a 5-HT agonist), 8-OHDPAT (a selective 5-HT_{1A} agonist), and fenfluramine (a 5-HT releasing agent); locomotor suppressant effects of m-CPP and prolactin responses to m-CPP than either Wistar or Sprague-Dawley (SD) rat strains. Isolated FH rats gained significantly less body weight relative to isolated Wistar or SD rats. These findings demonstrate that FH rats, a strain with a peripheral platelet storage pool disorder, also possess altered central nervous system serotonergic function.</p>		

Other Collaborative Professional Personnel Engaged on the Project

D.L. Murphy, M.D.	Chief	LCS	NIMH
R.M. Cohen, M.D., Ph.D.	Section Chief	LCM	NIMH
J. Zohar, M.D.	Guest Researcher	LCS	NIMH
K.M. Wozniak, Ph.D.	Visiting Associate	LCS	NIAAA
G. Bagdy, Ph.D.	Visiting Fellow	LCS	NIMH
J.L. Hill, Ph.D.	Biostatistician	LCS	NIMH
M. Haass, M.D.	Assoc. Professor	Dept. Cardiol., Univ. Heidelberg, W. Germany	
C.L. Devane, Ph.D.	Assoc. Professor	Dept. Pharmacy, Univ. Florida, Gainesville, FL	

Project Description

Brain serotonin (5-HT) changes have been implicated in the etiology of affective illness and the mode of action of antidepressant and antimanic drugs. Due to the therapeutic lag between the initiation of antidepressant treatment and onset of clinical effects, animal studies of molecular mechanisms pertinent to antidepressant efficacy have concentrated on the adaptive changes in various aminergic neurotransmitter systems following long-term antidepressant treatment. We have conducted a series of experiments to explore functional adaptational changes in the serotonergic neurotransmitter system regulating food intake, locomotor activity, temperature, and neuroendocrine changes following long-term administration of antidepressant drugs by using selective 5-HT subtype agonists as challenge agents. These adaptive changes might help us to understand the molecular mechanisms responsible for both the therapeutic and side effects of these drugs. In a separate series of experiments, we explored the possibility that fawn-hooded (FH) rats, a strain with a peripheral platelet 5-HT storage pool disorder, may also possess altered central nervous system serotonergic function with the hope that this strain may prove to be a useful animal model to probe brain serotonergic function.

Methods Employed

In the food deprived paradigm, the animals were trained to take their daily food (Purina food pellets) from 10:00 AM to 2:00 PM for 10 days before initiation of drug treatment. At the end of the first hour of food access, the remaining food was weighed; the difference from the original amount constituted one measure of food intake. In addition, daily (4 hours) food intake, 24-h water intake, and body weight gain were also recorded for each animal.

In the free-feeding paradigm, the animals were habituated to eating food pellets placed on cage floor (single cages) several times before saline or drug injection. On the day of the experiment, the rats were injected either with saline or various doses of drug and then immediately transferred to single cages containing a weighed amount of food on the floor. At the end of 2 hours, the remaining food was weighed; the difference from the original amount constituted the measure of food intake.

Locomotor activity of individual rats was recorded daily for a period of 30 min at the same time of the day in the same test cages (Coulbourn Instruments, 30 cm x 25 cm x 29 cm) each equipped with five photocell detectors that were located 6 cm apart and 2 cm above the grid floor. Interruptions of the photocell beams were recorded automatically by digital counters. Temperature was measured with a rectal probe (inserted 2.5 cm), with the rat loosely restrained by the tail; the reading was displayed on a digital thermometer. All measurements were made at room temperature ($25 \pm 1^\circ\text{C}$).

In neuroendocrine studies, for some experiments, the left femoral artery and vein were cannulated in each animal under halothane anesthesia and the catheters were exteriorized

subcutaneously at the back of the neck. Saline or various doses of 5-HT agonists were injected at least 48 hours after surgery. Blood samples were drawn from the femoral artery and collected in tubes containing EDTA. In other experiments, animals were sacrificed by decapitation and trunk blood was collected in centrifuge tubes containing EDTA. Following centrifugation, plasma samples were collected. Plasma concentrations of prolactin, corticosterone, and growth hormone were measured by radioimmunoassay.

In the antidepressant studies, clorgyline (1 mg/kg/day), imipramine (5 mg/kg/day), clomipramine (5 mg/kg/day), or saline was subcutaneously administered by means of osmotic minipumps for 28 days; the pumps were reimplanted after two weeks. Hypothalamic concentrations of 5-HT, norepinephrine, dopamine, and 5-hydroxyindoleacetic acid in different rat strains were determined by high pressure liquid chromatography. M-CPP concentrations in brain tissue were analyzed by high performance liquid chromatography (HPLC).

Major Findings

Administration of the 5-HT_{1A} receptor agonist, 8-OHDPAT, to rats produced dose-dependent decreases in food intake and hypothermia, increases in plasma prolactin (peak effect at 15 min) and corticosterone (peak effect at 30 min), and a decrease in plasma growth hormone (peak effect at 15 min) concentrations. Chronic (22 days) treatment with clorgyline attenuated the hypothermic response to 8-OHDPAT, whereas similar duration of treatment with the tricyclics, clomipramine and imipramine, did not significantly modify it. Acute treatment for 3 days with each of the antidepressants did not modify 8-OHDPAT-induced hypothermia. These findings suggest development of functional subsensitivity of the 5-HT receptors mediating the hypothermic response following chronic treatment with the MAO-inhibiting antidepressant clorgyline.

In another study, administration of fenfluramine to rats produced decreases in 1-hour food intake and locomotor activity. Short-term (2-6 days) or long-term (21-25 days) treatment with clorgyline potentiated fenfluramine-induced suppression of food intake but did not affect fenfluramine-induced suppression of locomotor activity. On the other hand, long-term but not short-term imipramine treatment attenuated fenfluramine-induced decreases in 1-hour food intake whereas neither short-term nor long-term imipramine treatment affected fenfluramine-induced decreases in locomotor activity. These findings demonstrate a differential effect of antidepressant treatment on fenfluramine-induced suppression of food intake and locomotor activity.

In a separate series of studies with the FH rat strain, FH animals were found to be significantly less sensitive to the food intake suppressant effects of 8-OHDPAT (a 5-HT_{1A} agonist), m-CPP (a 5-HT_{1B} agonist), and fenfluramine (a 5-HT releasing agent) than either Wistar or Sprague-Dawley (SD) rats. In the free-feeding paradigm, administration of various doses of 8-OHDPAT and buspirone (a 5-HT_{1A} agonist) produced significant increases in 2-hour food intake only in Wistar and SD strains and not in the FH strain. Similarly, various doses of m-CPP produced significant decreases in locomotor activity only in Wistar and SD strains and not in the FH strain. Isolated FH animals gained significantly less body weight relative to both Wistar and SD animals. In neuroendocrine studies, FH animal's prolactin responses to various doses of m-CPP were significantly smaller than that of either Wistar or SD rats, while corticosterone responses were equivalent across all three strains. On the other hand, baseline concentrations of corticosterone but not of prolactin were significantly higher in FH animals relative to both Wistar and SD animals. There was no significant difference in either baseline hypothalamic concentration of 5-HT, 5-HIAA, NE, or DA or brain concentrations of m-CPP among these three rat strains. These findings demonstrate that FH rats, a strain with a

peripheral platelet 5-HT storage pool disorder, also possess altered central nervous system serotonergic function.

Significance to Biomedical Research and Program of the Institute

The demonstration of functional adaptational changes in the serotonergic neurotransmitter mechanisms following long-term antidepressant drug treatment is important since this system is one of the two major neurotransmitter systems implicated in the etiology of affective illness. The differential effects of long-term antidepressant treatments on different 5-HT-mediated behavioral and neuroendocrine functions suggests that adaptational consequences of long-term treatment with various antidepressants are not equal throughout the brain and depend more specifically on changes induced within the brain areas influencing that particular paradigm, including changes in other interactive neurotransmitter systems. The demonstration of altered central nervous system serotonergic function in the FH rat strain is important since brain 5-HT changes have been implicated in the etiology of affective illness and mode of action of antidepressant and antimanic drugs. This rat strain may prove to be a useful genetic model to probe brain serotonergic function.

Proposed Course

During the next year, we will continue to explore functional adaptational changes in the serotonergic system using other 5-HT subtype agonists as challenge agents. We also plan to investigate changes in receptor densities in various 5-HT receptor subtypes following long-term treatment with the same antidepressants and to correlate these bindings changes with the functional changes observed. With the FH rat strain, we plan to identify other altered behavioral and neuroendocrine responses to serotonergic agents and clarify the biochemical nature of these defects.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00336-09 LCS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Phenomenology and Treatment of Obsessive-Compulsive Disorder in Adults

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Dennis L. Murphy, M.D., Chief, Laboratory of Clinical Science, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies conducted in the last year on obsessive-compulsive disorder (OCD) have had a primary focus on treatment of the disorder with clomipramine. Investigations conducted here and elsewhere have conclusively shown that this tricyclic drug is more effective than structurally similar tricyclics such as desipramine, imipramine, and amitriptyline. In contrast, in depression, panic disorder, and other psychiatric disorders, these agents have essentially identical efficacy. In a double-blind study attempting to evaluate what duration of clomipramine maintenance treatment might be most useful, we found that patients treated for 5 to 27 months all had a similar, very high relapse rate during placebo substitution for seven weeks. As all patients responded to clomipramine reinstatement, it appears that longer term maintenance treatment with this agent is useful. In studies evaluating possible serotonergic contributions to clomipramine's therapeutic effects, evidence was found that some brain serotonin subsystems (those mediating some behaviors and temperature) but not others (those mediating cortisol and prolactin responses) were down-regulated during clomipramine treatment of OCD patients.

Other Collaborative Professional Personnel Engaged on the Project

T.R. Insel, M.D.	Staff Physician	LCS	NIMH
C. Benkelfat, M.D.	Visiting Fellow	LCS	NIMH
M.T. Pato, M.D.	Guest Researcher	LCS	NIMH
T.A. Pigott, M.D.	Staff Fellow	LCS	NIMH
J.L. Hill, Ph.D.	Biostatistician	LCS	NIMH
G. Grover, R.N.	Nursing Staff	CC	NIH

Project DescriptionObjectives

1. To evaluate psychobiological features of adults with obsessive-compulsive disorder to better understand the development, course, and treatment of this psychiatric disorder.
2. In particular, to explore the possible contributions of changes in brain neurotransmitter and neuromodulator systems in obsessive-compulsive disorder symptoms.
3. To also evaluate the mechanism of action, pharmacokinetics, side effects, and other characteristics of drugs such as clomipramine that have therapeutic effects in obsessive-compulsive disorder.

Methods Employed

Patients are screened and, if suitable, accepted into our adult obsessive-compulsive disorder (OCD) clinic program. Diagnoses are established using DSM-III-R criteria based on a standardized interview schedule. Duration of illness, prior treatment, family history, and other relevant information is obtained. After stopping all treatment, we measure the patient's baseline symptom severity using a number of interview-based and self-rating scales. During this baseline, medication-free interval, patients are given a number of psychobiological tests. These tests include procedures to determine patient response to various neurotransmitter-selective pharmacological challenges such as m-chlorophenylpiperazine (m-CPP), a serotonergic agonist, caffeine, and naloxone. An LP may be performed to evaluate cerebrospinal fluid neurotransmitters, their metabolites and neuropeptides. Positron emission tomography under various conditions may also be performed.

Suitable patients are typically admitted into therapeutic trials comparing new drugs with other standard drugs such as clomipramine or placebo. Some of the psychobiological tests and ratings obtained at baseline are repeated at various times during drug treatment. Responses to treatment are compared with baseline measures and are correlated with changes in psychobiological measures and plasma levels of drugs.

Major Findings

When given to OCD patients, the serotonin agonist, m-CPP, unexpectedly elicited more marked behavioral responses, in contrast to the negligible behavioral changes observed in our initial studies in normal controls. Increased anxiety and an exacerbation of OCD symptoms were particularly noteworthy. The behavioral changes produced by m-CPP in the OCD patients were

different from the results with placebo or with the serotonin antagonist, metergoline, both of which were essentially inactive.

Increases in plasma prolactin and in temperature produced by m-CPP (which, according to animal studies, reflect m-CPP's serotonin agonist effects) were equivalent in OCD patients and normal controls, while plasma cortisol increases were significantly lower after m-CPP in the patient group compared to the controls.

In a follow-up study, we readministered m-CPP to a subset of OCD patients while they were receiving clomipramine. At this time, adverse behavioral responses to m-CPP were significantly attenuated, even though plasma concentrations of m-CPP were unexpectedly almost doubled when m-CPP was co-administered with clomipramine. As with the behavioral responses, the temperature increases produced by m-CPP were also attenuated during clomipramine treatment. The neuroendocrine (prolactin and cortisol) increases produced by m-CPP, however, were unchanged by clomipramine.

In a separate group of OCD patients, we evaluated the necessary duration of treatment with clomipramine. While clomipramine has been shown to be at least partially effective in the majority of OCD patients (according to the results of studies conducted here and elsewhere over the last 8 years), no previous study had assessed whether improvement would be sustained after discontinuation of clomipramine, or whether long-term, indefinite treatment was required. Twenty-one patients with OCD who had manifested sustained improvement during 5 to 27 months of clomipramine treatment agreed to participate in a double-blind discontinuation study. Of 18 patients who completed the study, 16 had a significant recurrence of OCD symptoms by the end of the 7-week placebo period. In addition, 11 patients developed a significant increase in depressive symptoms. Treatment duration prior to discontinuation was not related to the frequency or severity of increased OCD symptoms or to the appearance of depressive symptoms.

Significance to Biomedical Research and the Program of the Institute

The behavioral hyperresponsivity to the serotonin agonist, m-CPP, in OCD patients compared to controls is a novel observation. Behavioral hyperresponsivity in OCD patients has not previously been seen with single doses of other psychoactive drugs that have activating or anxiogenic properties, such as d-amphetamine or yohimbine. Thus, these results provide further evidence to support the hypothesis of an abnormality in the serotonin neurotransmitter system in patients with this disorder.

The finding that clomipramine treatment was associated with attenuated behavioral hypersensitivity to m-CPP is consistent with other data indicating that this and other serotonin-selective uptake inhibitors (such as fluoxetine and fluvoxamine) are more effective than other uptake inhibitors such as desipramine, imipramine, and amitriptyline, and that these therapeutic actions may depend, at least in part, in their capacity to down-regulate serotonergic functions.

At the same time, the information that prolactin and temperature responses to m-CPP were no different in OCD patients vs. controls, and that cortisol responsiveness was actually diminished in OCD patients indicates a heterogeneity in human central serotonin response systems. This finding is consistent with new animal data (including some from our laboratory), indicating that multiple serotonin receptors provide an opportunity for heterogenous and even opposing actions of different serotonergic agonists. The brain "serotonergic system" clearly functions not as a coordinated, monolithic entity, but as a set of separately acting subsystems. The

differential modulation of serotonin responses during clomipramine administration, which were observed in our studies, further support this concept.

The results of the clomipramine discontinuation trial suggest that prolonged drug treatment is necessary in clomipramine-responsive patients.

Proposed Course

We plan to continue exploring the question of serotonergic factors in OCD and in the mechanism of action of clomipramine by using other serotonin-selective agonists and antagonists, including a comparative treatment trial with a new serotonergic anti-anxiety drug. We will also study the effects of m-CPP and clomipramine using positron emission tomography techniques. At the same time, we plan to study m-CPP responses in other patient groups, and agents acting through other neurotransmitter systems in OCD patients, since it is unlikely that any complex disorder such as OCD is linked directly to a single brain neurotransmitter system. We will also study some new rating scales under development to find better symptom and diagnostic assessment procedures for this patient populations.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00337-09 LCS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropharmacology of Neuroendocrine and Neurotransmitter Regulatory Mechanisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Dennis L. Murphy, M.D., Chief, Laboratory of Clinical Science, NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch, NIMH; Neuroimmunology Branch, NINCDS

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.2

PROFESSIONAL:

1.4

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The functional status of the serotonin (5-HT) neurotransmitter system has been studied comparatively in humans and rhesus monkeys using 5-HT-selective agonists and antagonists as pharmacologic probes. Psychophysiologic responses, including blood pressure, neuroendocrine measures, temperature, penile erections, and subjectively assessed as well as objectively rated behavioral changes, are differentially altered by certain agonists, and in certain patient subgroups, as well as by the long-term administration of other psychoactive drugs such as tricyclic antidepressants, lithium, and monoamine oxidase inhibitors. For example, the 5-HT-selective agonist, m-chlorophenylpiperazine (m-CPP), and the 5-HT-releasing agent, fenfluramine, both affect prolactin, cortisol, and food intake, but only m-CPP elicits temperature and behavioral changes. M-CPP but not L-tryptophan was associated with headaches in individuals with a past history or family history of migraine. Many other examples support the concept that humans as well as other species have functionally different, independently modulated serotonergic subsystems which appear to correspond, at least in part, to the heterogeneous 5-HT binding sites and neuroanatomical subpathways identified *in vivo*.

Other Collaborative Professional Personnel Engaged on the Project

G. Bagdy, Ph.D.	Visiting Fellow	LCS, NIMH
T. Brewerton, M.D.	Assistant Prof.	Dept. of Psychiatry, Univ. South Carolina
N.A. Garrick, Ph.D.	Biologist	LCS, NIMH
P.W. Gold, M.D.	Section Chief	BP, NIMH
J.L. Hill, Ph.D.	Biostatistician	LCS, NIMH
D. Jimerson, M.D.	Assoc. Prof.	Dept. Psychiatry, Harvard Univ., Cambridge, MA
H.F. McFarland, M.D.	Section Chief	NIB, NINDCS
D.E. McFarlin, M.D.	Lab Chief	NIB, NINCDS
J.W. Rose, M.D.	Staff Physician	VA Salt Lake City, Utah
B.F. Roy, M.D.	Staff Physician	VA Washington, DC
T.P. Tomai	Chemist	BP, NIMH

Project DescriptionObjectives

The discovery that many newly characterized peptides and hormones are present in high concentrations in the brain, cerebrospinal fluid (CSF), and plasma has led to an entire field of inquiry into the interactions among peptides, hormones, and both the classical monoamine neurotransmitters as well as trace amines in brain. All these substances may function as modulators of neurotransmission. Overall, this project has focused on measuring various peptides, hormones, and several monoamines and their metabolites in CSF and plasma in an attempt to evaluate: (a) CNS and other physiologic influences on hormones and monoamines; (b) the relationship between peripheral, brain, and CSF neuropeptide levels; and, in particular, (c) the effects of drugs (especially agents with selective actions), as well as stress and other stimuli on monoamines, peptides, and hormones using biochemical, behavioral, neuroendocrine, and other physiologic response measures.

The principal focus of our work over the last few years has been to determine how to evaluate the apparent contributions of changes in brain serotonin (5-HT) function to neuropsychiatric disorders and to the effects of drugs active in treating these disorders. For strategic reasons discussed in a review article published in 1986, we moved from static, single point measures of 5-HT and its major metabolite, 5-hydroxyindole acetic acid (5-HIAA) in CSF, platelets, urine, or postmortem brain samples, to attempt to evaluate the state of functional responsiveness (and possibly receptor sensitivity) of the brain 5-HT system using drugs with 5-HT-selective actions as *in vivo* probes of the system.

Most of our investigations in humans are based on previous and ongoing studies exploring the neuroanatomy, physiology, and pharmacology of 5-HT in rodents and other species. Our group continues to use rodent and nonhuman primate models to test the validity of some of our so-called pharmacologic challenge studies in humans, particularly when more novel agents or dosage regimens are being studied (see Z01 MH 00332-10). However, there are some not unexpected neuroanatomical differences in the brain 5-HT systems between primates and rodents and, similarly, major species differences have become evident in the rapidly developing area of brain 5-HT receptors and binding sites. Thus, ultimately, 5-HT function needs to be studied directly in humans, particularly when, as some of the preliminary data indicate, disorder specific changes in responsivity to serotonergic agents occur.

When using pharmacological probes to study serotonergic responsivity in humans, we and others have used the 5-HT metabolic precursors, L-tryptophan and 5-hydroxytryptophan, reuptake inhibitors, such as clomipramine, fluoxetine, and fluvoxamine, the releasing agent, fenfluramine, and the 5-HT receptor agonists, meta-chlorophenylpiperazine (m-CPP), buspirone, and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OHDPAT), alone and together with some 5-HT antagonists such as metergoline, methysergide, and ritanserin.

Our single most studied agent is m-CPP, a metabolite of the antidepressant, trazodone, which was originally shown to act postsynaptically to elevate plasma prolactin via 5-HT_{1B} receptors in rodents. Our preliminary studies in rhesus monkeys demonstrated that m-CPP administration intravenously over a dose range of 0.5 to 3.0 mg/kg increased plasma prolactin, cortisol, and growth hormone, and was accompanied by a calming behavioral effect. After obtaining approval to conduct the first studies of m-CPP in humans, we demonstrated dose-related increases in plasma cortisol and prolactin, as well as a hyperthermic effect at a dose of 0.5 mg/kg given orally. Pretreatment with the 5-HT₁/5-HT₂ receptor antagonist, metergoline, prevented the m-CPP-induced increases in cortisol, prolactin, ACTH, and temperature, which was consistent with studies in rodents and monkeys that had previously shown metergoline to prevent m-CPP-induced behavioral and neuroendocrine changes. On the basis of this information, our group in collaboration with several other NIMH-IR groups have been investigating m-CPP's effects when given orally or intravenously in various patient groups and volunteers using a number of different physiologic end points.

Methods Employed

Human plasma is obtained from blood samples collected via indwelling venous catheters. Cerebrospinal fluid from nonhuman primates is collected by means of indwelling lumbar cannulae permitting continuous sampling through a refrigerated line into a fraction collector housed in a freezer. Plasma from the nonhuman primates and rodents is obtained by use of indwelling venous catheters that are usually implanted 15 to 24 hours prior to our studies, so that investigations can occur under non-stressful, basal conditions. Some examples of hormones measured by radioimmunoassay include cortisol, prolactin, growth hormone, β -endorphin, melatonin, ACTH, and vasopressin. Antibodies to β -endorphin, somatostatin, and other peptides are determined by enzyme-linked immunoabsorbent assay (ELISA). Serotonin and N-acetyl-5-HT are measured by high performance liquid chromatography with electrochemical detection (HPLC-EC). Other monoamines and monoamine metabolites as well as plasma m-CPP concentrations are also measured by HPLC-EC. Behavioral changes are assessed using a number of validated self-rating and interviewer's scales.

Major Findings

Our recent studies in normal human volunteers have shown that a small dose of m-CPP (0.1 mg/kg) given intravenously leads to equivalent increases in plasma prolactin and similar plateau concentrations of m-CPP in plasma as the larger, oral dose of m-CPP (0.5 mg/kg) used in our initial studies reported last year.

Minimal subjective or behavioral changes occurred in normal humans receiving 0.5 mg/kg m-CPP orally. In contrast, substantially greater increases in anxiety, dysphoria, and activation self-ratings following the intravenous administration of 0.1 mg/kg m-CPP. These findings contrast markedly to findings from our prior studies using moderately large single

doses (60 mg) of fenfluramine or L-tryptophan, which produced similar plasma prolactin elevations, but minimal behavioral changes.

In our initial studies in humans, we found that m-CPP given orally had negligible effects on blood pressure or heart rate. However, after administering intravenous m-CPP to rhesus monkeys or humans, we found elevations in systolic blood pressure, diastolic blood pressure, and heart rate. In rats, intravenously administered m-CPP produced dose-dependent blood pressure elevations, which were antagonized by metergoline and ritanserin, but not by adrenergic blockage using a combination of prazosin and yohimbine. M-CPP had similar effects in pithed, adrenal demedullated rats and in conscious, intact rats, indicating that m-CPP was apparently acting directly on the peripheral cardiovascular system. Some evidence of a differential behavioral sensitivity to m-CPP in psychiatric patient subgroups was found in patients with obsessive-compulsive disorder (OCD) receiving m-CPP under double-blind, placebo-controlled conditions experienced markedly greater anxiety and other behavioral symptoms than did normal controls. A striking but brief exacerbation of their obsessive-compulsive symptoms also occurred, as is described in greater detail in Z01 MH 00336-09 LCS. Treatment of these OCD patients with clomipramine attenuated the behavioral and temperature responses to m-CPP, but did not alter m-CPP's neuroendocrine effects. These results were consistent with studies in rodents that demonstrated that various psychoactive drugs including other tricyclics, lithium, and monoamine oxidase inhibitors, produced different patterns of changes in responses to 5-HT agonists like m-CPP and 8-OHDPAT.

An additional example of the sensitivity of certain patient groups to m-CPP's effects was an unexpected observation related to the vascular theory of migraine, which postulates an initial vasoconstrictor phase followed by the development of migraine headaches during a secondary phase of vascular dilatation. In a study in progress, we explored the possible anorexic effects of m-CPP in patients with bulimia given m-CPP orally. Initially, the most striking observation was an unusual frequency of headaches, many of the migrainous type. These headaches did not occur during the same time period as the immediate neuroendocrine, temperature, behavioral, or cardiovascular responses to m-CPP, but rather 8 to 12 hours after 0.5 mg/kg m-CPP administered orally. Headaches occurred significantly more often in patients with a personal or family history of migraine headaches than in those without such a history. Headache ratings were also significantly correlated ($r=0.70$, $p<0.001$) with peak concentrations of m-CPP in plasma. Since observing this side effect, we have avoided giving m-CPP to subjects with a migraine history. However, these observations indicate that m-CPP may provide a novel probe for studies of the pathophysiology of migraine headaches. Also, the possible usefulness of the cardiovascular stimulatory effect of m-CPP and similar agents may warrant further exploration because of their different mode of action from conventional sympathomimetic drugs.

One other effect of m-CPP deserves mention. Penile erections were regularly observed to follow intravenously administered m-CPP in our original neuroendocrine studies in rhesus monkeys, but not in humans given m-CPP orally. Further studies in monkeys revealed that this effect of m-CPP was also observed with other piperazine-type 5-HT agonists and with fenfluramine, but not with the 5-HT_{1A} agonists, 8-OHDPAT or buspirone. Similar observations have recently been made in rodents. M-CPP's parent compound, trazodone, is unusual among antidepressants since it is associated with priapism, an uncommon but noteworthy side effect that in some instances has required surgical intervention.

In other continuing studies of neuropeptides and antibodies to neuropeptides reported in greater detail in last year's annual report, we found a distinct circadian rhythm in corticotropin releasing hormone (CRH) in rhesus monkeys. This rhythm was over 12 hours out of phase with that of cortisol in CSF or plasma. While hypothalamic CRH is regarded as a major physiologic regulator of pituitary ACTH secretion and, thereby, of the circadian and

stress-related release of cortisol from the adrenal gland, CRH and CRH receptors are also widely distributed in other brain areas of primates and rodents. The marked difference in the circadian rhythm of CRH versus cortisol suggests that CRH in CSF reflects or mediates some non-hypophysiotropic brain functions of this peptide.

Antibodies to two peptides, β -endorphin and somatostatin, were identified and characterized last year in human plasma. In studies of psychiatric patients, 6 of 10 (60%) individuals with major depressive disorder demonstrated antibodies reactive with somatostatin 1/14 in contrast to 1/15 (7%) controls. Overall, anti-somatostatin reactivity was significantly higher in patients with major depressive disorder (0.233 ± 0.177) than in the normal volunteers (0.04 ± 0.039 , $p < 0.01$). Anti-somatostatin IgG was isolated by affinity chromatography. The recognition site for somatostatin was retained by F(ab')₂ fragments. In other continuing studies of antibodies to β -endorphin, we found sera from 2 patients with major depressive disorder to have anti-idiotypic antibodies which specifically inhibited reactivity between anti- β -endorphin immunoglobulin G (IgG) and β -endorphin. Autologous and homologous anti-idiotypic anti-anti- β -endorphin IgG antibodies were isolated by affinity chromatography. The purified anti-idiotypic antibody did not bind β -endorphin but competed with [¹²⁵I] β -endorphin for rat brain opiate receptors. Normal IgG which was similarly treated had negligible competitive effects. Binding of the anti-idiotypic to a 60,000 Dalton protein from rat brain was detected by Western immunoblot analysis. This protein corresponds in molecular weight to proteins proposed to be components of opiate receptors. While there has been little prior exploration of the existence of antibodies to endogenous neuropeptides, such antibodies could be relevant to neuropsychiatric and other human disorders.

Significance to Biomedical Research and the Program of the Institute

The present results indicate that m-CPP and other agents believed to act through central and/or peripheral serotonergic mechanisms have behavioral, neuroendocrine, cardiovascular, and other physiological effects in humans, which, in general, resemble those seen in other species, especially rodents and nonhuman primates. Limited antagonist studies in humans suggest that many of m-CPP's central effects are most likely to be mediated via 5-HT₁ sites; evidence from animal studies indicates that m-CPP may also possess cardiovascular action mediated by 5-HT₂ sites, and also bind to some other neurotransmitter sites which are located both peripherally and centrally.

Overall, these studies support the hypothesis that functionally distinct brain 5-HT subsystems exist in humans which correspond, at least in part, to the heterogeneous 5-HT binding sites and neuroanatomical subpathways identified *in vitro*. These studies also provide a basis for the comparative evaluation of different physiologic responses, which can be used to investigate the status of these different 5-HT subsystems in human disorders and as they may be affected by psychotherapeutic drug treatment.

Proposed Course

Based on our studies with m-CPP and other 5-HT agonists and antagonists in rodents, monkeys, and humans, we have begun to use these agents as probes to evaluate possible abnormalities in 5-HT function in various psychiatric disorders. Collaborations employing these agents have been established with other groups within NIMH to evaluate patients with eating disorders, panic disorder, alcoholism, borderline personality disorder, and in other NIH institutes and elsewhere to evaluate possible antinociceptive and cardiovascular effects of these serotonergic subsystem probes. In regard to the neuropeptides, investigations by Dr. Roy are underway regarding the occurrence of antibodies to other peptides in human plasma and their

relationship to additional neuropsychiatric disorders besides depression, such as Alzheimer's disease and obsessive-compulsive disorder. Additional pharmacologic studies should be important in gaining a better understanding of drug and disease-related changes in 5-HT function in humans.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00339-07 LCS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropharmacology of Cognition and Mood in Geriatric Neuropsychiatry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Trey Sunderland, M.D., Chief, Unit on Geriatric Psychopharmacology, LCS, NIMH

COOPERATING UNITS (if any)

Laboratory of Cerebral Metabolism, NIMH; Biological Psychiatry Branch, NIMH;
Medical Neurology Branch, NINCDS

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:

6

PROFESSIONAL:

3.5

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The pharmacologic challenge model continues to be the major research strategy of the Unit on Geriatric Psychopharmacology. By using drugs to investigate underlying biologic function, we are testing potential diagnostic methods, while simultaneously attempting to develop new drug treatments. This past year, we concluded major studies on Alzheimer's disease, a condition with known cholinergic neuropathology, with two cholinergic agents, arecoline and nicotine. These studies follow our earlier findings of higher functional sensitivity of Alzheimer patients than normal controls or elderly depressives to the anticholinergic agent, scopolamine, which suggest that the cholinergic system in Alzheimer's disease still responds to exogenous drug manipulations. These findings have also led to the development of our latest pilot studies with non-cholinergic medications such as the serotonin agonist, m-chlorophenylpiperazine (m-CPP), and the neuropeptide, thyrotropin-releasing hormone (TRH).

With the high degree of overlapping symptoms between geriatric depression and dementia, it is essential to evaluate patients carefully. Consequently, we have systematically compared and contrasted these two major geriatric disorders to ensure diagnostic accuracy and to understand better the common pathologic mechanisms. Given the lack of definitive diagnostic markers, we developed special rating scales to measure depression and daily functioning in dementia subjects. Biological specimens such as cerebrospinal fluid have also been tested to help differentiate depressed and demented patients from age-matched controls. In addition, extensive cognitive testing provides us with an important profiling tool. Together, these instruments allow us to assess the effectiveness of our ongoing medication studies and will enable us to continue our correlative and longitudinal studies in the future.

Other Collaborative Professional Personnel Engaged on the Project

D.L. Murphy, M.D.	Chief	LCS	NIMH
H. Weingartner, Ph.D.	Guest Researcher	BPB	NIMH
P.A. Newhouse, M.D.	Guest Researcher	LCS	NIMH
R.M. Cohen, M.D., Ph.D.	Section Chief	LCM	NIMH
M. Gross, M.D.	Staff Psychiatrist	LCM	NIMH
B.A. Lawlor, M.D.	Staff Psychiatrist	LCS	NIMH
S.E. Molchan, M.D.	Staff Psychiatrist	LCS	NIMH
A.M. Mellow, M.D., Ph.D.	Staff Psychiatrist	LCS	NIMH
J. Grafman, Ph.D.	Staff Psychologist	MNB	NINCDS
R. Martinez, M.D.	Staff Psychiatrist	LCS	NIMH

Project DescriptionObjectives

1. To develop innovative pharmacologic strategies in Alzheimer's disease and geriatric depression. Since there is currently no standard treatment for dementia, there are many aspects of drug treatment that have yet to be explored. We have previously demonstrated short-term benefits in Alzheimer patients with the monoamine oxidase inhibitor, L-deprenyl, and are now engaged in long-term L-deprenyl studies. Other potential drug treatments must also be investigated, so we are testing the serotonin agonist, m-chlorophenylpiperazine (m-CPP), and the peptide, thyrotropin-releasing hormone. Ultimately, we would like to combine experimental agents to find synergistic effects in Alzheimer subjects. In elderly depressed subjects, we are also testing the new therapeutic agents, L-deprenyl and m-CPP, to find more selective and less toxic antidepressant regimens.

2. To characterize the biological changes occurring in Alzheimer's disease and compare them to those of age-matched controls and depressives. To help differentiate Alzheimer's disease from geriatric depression, we are comparing Alzheimer and depressed patients using a multitude of variables including cerebrospinal fluid (CSF) markers, urine metabolites, neuroendocrine responses, brain imaging techniques, and cognitive testing. While some of these measures will separate Alzheimer and depressed patients, other variables [i.e., CSF somatostatin and dexamethasone suppression test (DST) response] show remarkable similarities, suggesting biological as well as clinical overlap between depression and dementia. Therefore, we would like to use each illness as a possible foil for the other and increase the areas of comparison. Whenever possible, we will also follow the variables longitudinally to help expand our understanding of the complex underlying biologic mechanisms of depression and dementia.

3. To explore the functional significance of the cholinergic deficit in Alzheimer's disease. While the cholinergic abnormalities in Alzheimer's disease have been widely documented in research on dementia, the therapeutic repercussions of this deficit have thus far been limited. By testing Alzheimer patients with cholinergic agonists and antagonists, we are evaluating potential new treatments (i.e., nicotine and arecoline) while also exploring the underlying mechanisms of the cholinergic impairments. The increased functional sensitivity of Alzheimer patients to scopolamine challenge allows us now to compare them to other diagnostic groups (i.e., Parkinson's patients and Korsakoff patients). In addition, we can test subjects at risk for Alzheimer's disease to determine whether scopolamine responsivity could be used as a pharmacologic diagnostic marker or indicator of therapeutic responsivity.

Methods Employed

Diagnostic Criteria. The final diagnosis of Alzheimer's disease can only be confirmed neuropathologically by biopsy or autopsy. Otherwise, the best estimates for the accuracy of clinical diagnosis are approximately 80-90% correct. Thus, to maximize the accuracy of the clinical evaluation, we test patients with multiple assessment instruments including the Clinical Dementia Rating (CDR) scale of Hughes and coworkers. Strict adherence is paid to the generally accepted DSM-III-R and ADRDA-NINCDS diagnostic criteria. Diagnosis of major affective disorder in elderly subjects is determined using DSM-III-R criteria. Subjects also undergo extensive neuropsychological testing and medical examinations to exclude those with confounding cognitive syndromes or serious medical complications. Autopsy specimens are currently being collected to confirm the diagnosis of Alzheimer's disease wherever possible.

Behavioral and Psychological Assessment. Alzheimer patients frequently do not understand or in some cases misinterpret questions on traditional rating scales of mood and behavior. Consequently, we developed several rating instruments specifically for Alzheimer patients. For example, we created the Dementia Mood Assessment Scale (DMAS) to measure mood changes in these subjects when we found that the standard Hamilton Depression Rating Scale (HDRS) was not appropriate for Alzheimer patients. Ability to perform activities of daily living is also an important measure of functional change in Alzheimer's disease. Once more, we developed a specific rating instrument to begin assessing these skills at baseline and continue longitudinally with input from nursing staff, family, and occupational therapist. Other scales include the modified global (15-point) ratings, Brief Psychiatric Rating Scale (BPRS), and various objective visual analog scales. Elderly depressed subjects are evaluated with more traditional objective and subjective ratings such as the Beck Depression Inventory, HDRS, BPRS, Profile of Mood States, and global rating scales.

Neuropsychological assessment of the geriatric depressed patients and Alzheimer patients involves a large number of standard and experimental testing paradigms. To establish common ground with other research centers studying Alzheimer patients, we test patients with several widely used rating scales such as the Wechsler Memory Quotient, Minimental State Examination, Mattis Dementia Rating Scale, and Boston Naming tests. Patients also undergo exploratory testing of semantic and recognition memory, free recall, vigilance, and attention. Increased emphasis and new experimental paradigms are now being developed for areas of procedural learning and visual memory.

Biological Assessment. To further exclude other causes of dementia or depression, we obtain multiple biological samples from subjects for research testing. These samples include plasma, platelets, urine, and CSF to measure hormone, monoamine metabolite, and peptide levels. Various neuroendocrine tests including the TRH stimulation test and DST are also used. Clinical brain imaging techniques including the CT scan, MRI, and mapping EEG are administered. Selected study participants also contribute skin samples or lymphocytes for further biochemical and genetic analysis.

After the initial baseline evaluation, patients are invited to participate in a series of pharmacologic challenge tests. Patients and normal controls are given intravenous or oral medications (i.e., scopolamine, nicotine, arecoline, lorazepam, m-CPP, or TRH) and monitored for several hours following drug administration for physiologic, behavioral, neuroendocrine, and cognitive changes, which are compared to results after placebo administration. The final phase of biological assessment includes short-term and long-term medication trials. Patients can participate in one or more trials that last from eight weeks to two years. During these studies, many of the same biological tests are repeated to help assess the pharmacologic and therapeutic effects of these medications.

Major Findings

We were the first group to document that Alzheimer patients are more sensitive than age-matched controls to the behavioral and cognitive effects of the anticholinergic agent, scopolamine. We have now demonstrated that this same pattern of increased functional sensitivity to scopolamine is not found in elderly depressed subjects. Such pharmacologic differentiation is important because there is frequent diagnostic confusion between Alzheimer's disease and geriatric depression, especially since depression is complicated by concomitant cognitive impairment (i.e., pseudodementia). We have shown that these functional changes in Alzheimer patients are not secondary to the sedative effect of scopolamine, by documenting that the benzodiazepine, lorazepam, does not produce the same cognitive effects as scopolamine despite causing similar sedation.

Therapeutically, considerable pilot work was completed this year with four major medications. Pursuing our previous discovery of increased functional sensitivity to the cholinergic antagonist, scopolamine, we tested two postsynaptic cholinergic agonists, nicotine and arecoline. Both drugs were tested in separate dose-response studies with Alzheimer patients. With different doses of nicotine, we noted surprisingly marked affective sensitivity with relatively little cognitive change. Arecoline, on the other hand, produced modest cognitive improvement in picture recognition without significant affective change, suggesting different cholinergic mechanisms for cognitive effects in Alzheimer patients. Pilot studies were also completed with the neuropeptide, TRH, and the serotonergically active trazodone metabolite, m-CPP. Both studies revealed that the TRH and m-CPP were well-tolerated in elderly subjects, which suggests that further studies of these agents in Alzheimer patients can now be conducted.

The relationship between depression and dementia continues to warrant both clinical and biochemical investigation. In the past year, we demonstrated that somatostatin, a neuropeptide known to decrease in Alzheimer's disease, also decreases similarly in the CSF of elderly depressed patients. To improve the clinical assessment of depression in demented patients, we developed a reliable rating instrument specifically designed to measure mood in Alzheimer patients. Our work with rating scales has also led to the development of an experimental assessment of activities of daily living in demented subjects that allows us to quantify this important measure of functioning.

With the recent discovery of a possible gene for Familial Alzheimer Disease (FAD) on chromosome 21, there has been an increased interest in the genetics of Alzheimer's disease. We have now completed the largest twin study to date of Alzheimer patients and found that only 40% of twin pairs were concordant for Alzheimer's disease at the time of study. While the concordance rate is expected to increase in this cohort over time as subjects get older, this study suggests that some undetermined environmental or other non-genetic factors may also affect the phenotypic expression of Alzheimer's disease.

Significance to Biomedical Research and the Program of the Institute

A perplexing question in the treatment of Alzheimer patients is why cholinergic replacement drugs are not more successful, especially since the brains of Alzheimer patients have been shown to have cholinergic deficits. By testing the functional sensitivity of Alzheimer patients to brief pharmacologic challenges with the anticholinergic agent, scopolamine, we have demonstrated three important findings. First, the group differences between demented, depressed, and elderly normal controls has potential diagnostic utility (i.e., scopolamine could be used as a pharmacologic probe in the absence of the definitive antemortem diagnostic

markers). Secondly, the increased functional responsivity of Alzheimer patients to scopolamine suggests that certain patients might show increased responsiveness to other cholinergic agents, particularly the postsynaptic agonists, nicotine and arecoline. Finally, the dementia-like effect created by scopolamine in elderly normal controls could provide a transient pharmacologic model for Alzheimer's disease that could be used as a human model for new drug testing.

While the cholinergic hypothesis has dominated the approach to treatment of Alzheimer's disease in most centers around the world, our program has purposely branched out in several new areas. Following preliminary success with short-term use of the monoamine oxidase inhibitor, L-deprenyl, we are currently studying the long-term effects of L-deprenyl in Alzheimer patients. In addition, the serotonin agent, m-CPP, is being tested as a pharmacologic probe with potential as a therapeutic agent. Perhaps most significantly, the neuropeptide, TRH, is being administered to Alzheimer patients in doses never before given to humans with this disease. Even if only a fraction of the drug crosses the blood-brain barrier, there should be enough of a central effect that TRH alone or in combination with other agents may ultimately improve neurotransmitter function and lead to therapeutic benefit.

One unexpected benefit of the continued interest in the depression syndrome of Alzheimer's disease has been the development of new rating instruments to quantify better the clinical status of Alzheimer patients. A rating scale of mood in demented patients as well as an experimental paradigm to measure activities of daily living functioning have already generated great interest from other researchers and are currently being adapted for use in multiple centers around the country. Other rating instruments are also currently being developed. This methodology has led to careful quantification of function and behavior in our ongoing investigations of antidepressant medications in these patients. With the possibility of immediate clinical benefit, these studies will also generate important information about the biochemical and clinical changes that occur over time in depressed and non-depressed Alzheimer patients and thus lead to future therapeutic strategies.

Proposed Course

Our continued interest in the sensitivity of Alzheimer patients to anticholinergic challenge with scopolamine will lead us in several directions. First, we are expanding the number of dementia patients tested with scopolamine to examine the questions of diagnostic specificity and underlying mechanism of action. Second, we are beginning to explore the use of scopolamine challenge as a predictive marker in at-risk subjects and as a prognostic test in early Alzheimer patients. This aspect of our study will require long-term follow-up. Third, we will be using the scopolamine model of dementia to test new therapeutic approaches to Alzheimer's disease.

Improving therapeutics for the memory and behavioral disorders of demented and depressed patients continues to be the central goal of the Unit on Geriatric Psychopharmacology. To achieve this goal, we are developing a coordinated system of acute, short-term and long-term treatment protocols to test the potential usefulness of new pharmacologic agents. Agents currently being studied are several cholinergic (nicotine and arecoline) and non-cholinergic agents (m-CPP, TRH, and L-deprenyl). It is likely that no single agent alone will reverse the symptoms of Alzheimer's disease. Therefore, we are testing agents (i.e., deprenyl) that may alter the course of the illness without necessarily reversing the symptoms and are developing "combination drug strategies." It may be discovered, for instance, that neurotransmitter-selective treatments have beneficial synergistic effects when used in combination. In addition, this strategy will eventually allow us to examine neuropeptide modulation of neurotransmitter function in humans, an important step to understanding the biochemical control of brain function.

The comparative study of depression and dementia will continue to provide insights into both of these illnesses. In addition to studying behavioral and neuroendocrine similarities, we will now be studying the biological markers including mapping EEG, CT scan, CSF metabolites, and plasma catecholamines. The important areas of cognitive overlap between demented and depressed patients will also be studied and compared further to age-matched controls. While depression in elderly patients has often been noted to mimic dementia (i.e., pseudodementia), it may be that dementia can also mimic depression, both behaviorally and biochemically. If this is the case, the longitudinal study of Alzheimer patients with careful correlative studies and profiling of clinical and biologic measures may provide us with important insights to the underlying mechanisms of both dementia and geriatric depression.

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Sills MS, Mellow AM, Sunderland T, Aulakh CS, Newhouse PA, Jacobowitz DM. Effect of chronic administration of scopolamine on thyrotropin-releasing hormone (TRH)-mediated wet-dog shakes and TRH receptors as measured by quantitative autoradiography, *Ann NY Acad Sci*.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02429-01 LCS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of CNS Opioid Receptors and Psychotomimetic Binding Sites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Richard B. Lofthman, M.D., Ph.D., Chief, Unit on Receptor Studies, LCS, NIMH

COOPERATING UNITS (if any)

Section on Drug Design and Synthesis, LN, NIDDK; Walter Reed Army Inst. Res., Washington DC;
Unit on Functional Neuroanatomy, BPB, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:

3

PROFESSIONAL:

1.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A major focus of work is the study of CNS opioid receptors. A goal of this work is to further define opioid receptor subtypes and develop new small molecules as research tools. Using site-directed acylating agents and receptor autoradiography, the opioid receptors labeled by (-)-[³H]cycloFOXY, an opiate antagonist suitable for positron emission tomography (PET), were shown to be μ and not δ receptors. (+)CycloFOXY was shown not to interact with opioid, phencyclidine, or σ receptors, supporting the use of ¹⁸F-(+)-cycloFOXY to estimate "nonspecific" binding in PET studies. Using the site-directed alkylating agent, β -funaltrexamine, we obtained autoradiographic data to support the existence of two μ binding sites in rat brain. [³H]-6- β -fluoro-6-desoxy-oxymorphone ([³H]-FOXY) was shown to label selectively and to be a high yield photoaffinity probe for μ opioid receptors. The peptide, MeTyr-D-Ala-Gly-N(Et)-CH(CH₂-Ph)CH₂-N(CH₃)₂ (LY164929), was shown to be highly selective for the lower affinity [³H]D-ala²-D-leu⁵enkephalin binding site. Nor-binaltorphimine, a selective κ antagonist in vivo, was shown to be about 20-fold selective for κ binding sites in vitro. An unexpected finding was the high levels of opioid peptides in rat brain membranes. Studies of morphine tolerance demonstrated that chronic morphine administration upregulated a μ binding site labeled by [³H]cycloFOXY and that chronic morphine and chronic naltrexone upregulated opiate receptors by different mechanisms. Manipulation of fluid balance was shown to regulate physiologically neurohypophyseal κ opiate receptors. Chronic drug abuse was shown not to alter psychotomimetic binding sites in human frontal cortex. Chronic administration of opiate agonists and antagonists and haloperidol were shown to alter rat brain phencyclidine (PCP) receptors. Studies of the interaction of enantiomeric pairs of unnatural opiates with PCP receptors led to the identification of (+)-pentazocine as a potential PET ligand for the σ receptor.

Other Collaborative Professional Personnel Engaged on the Project

M. Herkenham, Ph.D.	Chief, UFN	CNE	NIMH
L. Brady, Ph.D.	Senior Staff Fellow	CNE	NIMH
S. McLean, Ph.D.	Senior Staff Fellow	CNE	NIMH
K.C. Rice, Ph.D.	Chief, DDS	LN	NIDDK
A.E. Jacobson, Ph.D.	Research Chemist	LN	NIDDK
B. de Costa, Ph.D.	Fogarty Fellow	LN	NIDDK

Project DescriptionsProject 1. Characterization of CNS Opioid Receptors

1A. Development of small molecules as research tools. This work, conducted in collaboration with Dr. Rice, involves the *de novo* development of new agents as well as characterization of potentially useful chemicals synthesized in other laboratories. These agents include site-directed alkylating agents, radioligands, and drugs that are selective for receptor subtypes.

6- β -fluoro-6-desoxy-oxymorphone (FOXY) is a fluorinated derivative of oxymorphone originally developed as a potential positron emission tomography (PET) scanning ligand. Preliminary work (Rothman et al., *Neuropeptides* 4:311-317, 1984) demonstrated that [3 H]FOXY selectively labeled μ opioid binding sites with low levels of nonspecific binding. In this study the opiate receptor subtypes labeled by [3 H]FOXY and [3 H]D-al 2 -MePhe 4 -Gly 5 -enkephalin ([3 H]DAGO) were compared using site-directed acylating agents and binding surface analysis. Although the data indicated that both ligands selectively label μ opiate receptors, other experiments demonstrated that [3 H]DAGO and [3 H]FOXY labeled μ binding sites differently. Additional experiments demonstrated that [3 H]FOXY can be used as a high yield photoaffinity label for the μ opiate receptor subtype.

Most radiolabeled ligands used to label opiate receptors bind to multiple binding sites. Subtype-selective ligands make it possible to label single sites by "block" binding the radiolabeled ligand to selected subtypes. This study compared the selectivity of several ligands for the higher and lower affinity [3 H]D-al 2 -D-leu 5 -enkephalin binding sites. The results demonstrated that while morphine and DAGO were 80- and 256-fold selective for the lower affinity [3 H]D-al 2 -D-leu 5 -enkephalin binding site, LY164929 was 1,986-fold selective. Additional experiments indicated that whereas morphine was a noncompetitive inhibitor at the lower affinity [3 H]D-al 2 -D-leu 5 -enkephalin binding site, LY164929 was a competitive inhibitor, suggesting that this peptide might exhibit different properties *in vivo* than that of other μ -like ligands.

SuperFIT is an high affinity acylating ligand derived from fentanyl. Previous studies have suggested that a selective acylation of δ receptors resulted by exposing membranes to this and to other structurally related compounds. We found in a preliminary study that intracerebroventricular administration of either superFIT or its enantiomer 18 to 24 hours prior to sacrifice decreased the subsequent binding of [3 H]DADL to both higher and lower affinity binding sites.

Norbinaltorphimine (nor-BNI) is a bifunctional reagent developed as a selective antagonist of the κ opioid receptor. In another study, we examined the *in vitro* selectivity of nor-BNI, 6-desoxy-6 β -fluoronaltraxone (cycloFOXY), and the enantiomer of cycloFOXY ((+)-cycloFOXY),

among opioid receptor subtypes. Nor-BNI exhibited the highest affinity for κ binding sites labeled by [^3H]-U69,593 ($K_i=1.8$ nM) and was 27- to 29-fold less potent at μ and δ binding sites. In contrast, cycloFOXY had the highest affinity for μ binding sites ($K_i=2.62$ nM) and bound to κ and δ binding sites with K_i 's of 9.3 nM and 89 nM, respectively. The enantiomer of cycloFOXY did not inhibit binding even at concentrations greater than 10 μM , validating in part the use of ^{18}F -labeled (+)-cycloFOXY to estimate "nonspecific binding" in PET scans. Additionally, we report that (*S,S*)-U50,488 and (*R,R*)-U50,488 bind to κ binding sites labeled by [^3H]-U69,593 with K_i 's of 0.89 nM and 299 nM, respectively.

In another study, the enantiomers of U50,488, a ligand highly selective for the κ opioid receptor, were prepared; absolute configurations of these enantiomers were determined. Binding data demonstrated that the apparent dissociation constants for a κ binding site were 124 nM and 90,300 nM for the (1*S*,2*S*)-U50,488 and (1*R*,2*R*)-U50,488 enantiomers, respectively.

1B. Studies of opioid receptor subtypes. This project, conducted in collaboration with Dr. Rice, uses quantitative ligand binding, site-directed alkylating agents, and receptor autoradiography to help us understand further the multiple types of opioid receptors. Previous studies support the hypothesis of an opiate receptor complex consisting of adjacent and interacting μ and δ binding sites, and a δ binding site not associated with the receptor complex. Based on several lines of evidence, we hypothesized that two classes of μ binding sites exist: one part of the complex called the " μ_{CX} binding site," and another that is not part of the complex called the " μ_{NCX} binding site." Other studies were performed to determine the opioid receptor subtypes labeled by [^3H]cycloFOXY, an opioid antagonist suitable for PET studies.

Previous studies demonstrated that pretreatment of brain membranes with the irreversible μ antagonist, β -funaltrexamine (β -FNA), partially eliminated μ binding sites (Rothman et al., Eur. J. Pharmacol. 95:147-148, 1983; Tam and Liu-Chen, J. Pharmacol. Exp. Ther. 239:351-357, 1986), which was consistent with the existence of two μ binding sites distinguished by β -FNA. This study tested the hypothesis that the FNA-sensitive and FNA-insensitive μ binding sites have different anatomical distributions in rat brain. Prior to autoradiographic visualization of μ binding sites, [^3H]oxymorphone, [^3H]DAGO, and [^{125}I]D-al 2 -MePhe 4 -met(o)-ol-enkephalin (FK33824) were shown to label selectively μ binding sites using slide mounted sections of molded and minced rat brain. As with membranes, β -FNA eliminated only a portion of μ binding sites. Autoradiographic visualization of μ binding sites using the μ -selective ligand, [^{125}I]FK33824, in control sections and FNA-treated sections of rat brain demonstrated that the proportion of μ binding sites sensitive to β -FNA varied across regions of the brain, particularly the dorsal thalamus, ventral basal thalamus, and hypothalamus, providing anatomical data supporting the existence of two classes of μ binding sites in rat brain.

The autoradiographic distribution of [^3H]cycloFOXY after *in vivo* administration or *in vitro* incubation with sections of rat brain suggests that [^3H]cycloFOXY labels μ and κ opioid receptors. In the rat, the pattern of [^3H]cycloFOXY binding is similar to the distribution of μ receptors; however, labeling is also present in the neural lobe of the pituitary, central nucleus of the amygdala, and the hypothalamus, areas where κ receptors outnumber μ receptors. In the guinea pig, [^3H]cycloFOXY binding sites are dense in the deep layers of the cortex, an area enriched in κ receptors.

In another study, using the quantitative ligand binding method "binding surface analysis," *in vitro* autoradiography, and site-directed alkylating agents, we found that both [³H]cycloFOXY and [³H]naloxone labeled μ and κ , but not δ opioid receptors *in vitro*. The relevance of the results were discussed from clinical and basic science perspectives.

In another study, possible changes in the molecular weight of μ and δ opioid receptors during development were determined using cross-linked ¹²⁵I- β -endorphin. In adult rat brain, ¹²⁵I- β -endorphin labeled a 65 kD band that was eliminated when the membranes were pretreated with the μ -selective site-directed alkylating agent, BIT, but not with the δ -selective alkylating agent, FIT. In neonates, the single band labeled by ¹²⁵I- β -endorphin shifted to a MW of 54 kD. Ligand binding studies in neonates demonstrated only one binding site that could be labeled only by the μ -selective ligand, [³H]DAGO. These data are consistent with the hypothesis that the MW of the μ receptor changes with development and that these changes are possibly a result of glycosylation.

1C. Mechanisms of morphine tolerance and dependence. Despite decades of research on what causes tolerance and dependence to opiates, the mechanism is still unknown. Numerous early studies investigated the hypothesis that chronic administration of morphine altered opioid receptors. Taken as a whole, the results were equivocal. Most of these investigators reported no change in either affinity or number of binding sites. However, these early studies were conducted before it was recognized that opioid receptors had subtypes and these studies used radiolabeled ligands, such as [³H]naloxone and [³H]dihydromorphine, which are now known to label more than one binding site. Thus, these studies were not designed for the possibility that chronic morphine might alter only one subtype of binding site labeled by a [³H]ligand. Studies conducted in our laboratory using [³H]D-ala²-D-leu⁵-enkephalin support this hypothesis. These studies demonstrated that the chronic administration of morphine selectively upregulated only one of the two binding sites labeled by [³H]DADL (the lower affinity binding site), which marks the δ binding site of the opiate receptor complex. We have extended these initial studies by comparing the opioid binding sites upregulated by chronic morphine with those upregulated by chronic naltrexone, and have demonstrated that chronic morphine upregulates a μ binding site labeled by [³H]cycloFOXY.

In one study, we examined the hypothesis that chronic morphine and chronic naltrexone upregulate [³H]D-ala²-D-leu⁵-enkephalin binding sites by different mechanisms. In this experiment, rats were chronically administered either morphine or naltrexone. Chronic morphine upregulated the lower affinity, while chronic naltrexone upregulated both the higher and lower affinity [³H]D-ala²-D-leu⁵-enkephalin binding sites. Unlike the lower affinity [³H]D-ala²-D-leu⁵-enkephalin binding sites found in membranes prepared from placebo-pelleted rats, the lower affinity binding sites upregulated by naltrexone and morphine were partially (naltrexone) or completely (morphine) labile to preincubation for 60 min at 25°C in 50 mM Tris-HCl, pH 7.4, containing 0.4 M NaCl. These data suggest that chronic morphine and chronic naltrexone upregulate [³H]D-ala²-D-leu⁵-enkephalin binding sites via different mechanisms, and that the lower affinity [³H]D-ala²-D-leu⁵-enkephalin binding sites upregulated by chronic morphine and chronic naltrexone might differ biochemically from the lower affinity binding sites found in placebo membranes.

In a second study, we examined the effect of chronic administration of morphine on μ and κ binding sites labeled by [³H]cycloFOXY. We reported additional evidence that [³H]cycloFOXY

labeled two binding sites in rat brain membranes, identified as μ and κ , and demonstrated that the chronic administration of morphine selectively upregulated the μ binding site. The implications of these findings for models of the opioid receptors and the sodium effect were discussed.

To rule out the possibility that the changes observed in opioid receptor levels following chronic administration of morphine were caused by masking, or unmasking of binding sites by endogenous opioid peptides, we used RIAs to measure directly the levels of dynorphin-A(1-17), CCK-8, and met-enkephalin-arg-gly-leu (MERGL) in lysed-P2 membranes. Lysed-P2 membranes contained peptides at levels between 0.253 and 1.727 pmol/mg protein. The stability of the peptides during prolonged incubations at 25°C suggested that they were sequestered. Preincubation of membranes in 0.4 M NaCl greatly reduced the peptide levels, without altering [3 H]etorphine binding, suggesting that the sequestered peptides are not bound to opioid receptors, and, therefore, unlikely to affect the results observed in our chronic morphine studies.

Project 2. Studies of CNS psychotomimetic binding sites

Psychotomimetic binding sites can be defined as CNS binding sites/receptors that mediate or are involved in the genesis of psychotic-like symptoms/signs in human beings. In addition to amine receptor systems, such as the dopaminergic and serotonergic systems, that are thought to mediate psychotomimetic effects under certain conditions, there is evidence that the haloperidol-sensitive " σ " binding site and phencyclidine (PCP) binding sites might be involved in psychotomimesis in human beings. Although their structure-activity profiles and anatomical distribution binding sites differ, PCP and σ have the same high affinity for certain benzomorphan opiates, both natural and unnatural. Our initial studies of PCP and σ receptors were done to determine their response to chronic drug treatments.

In one study, haloperidol was administered via osmotic minipumps (0.24 mg/kg/24 hr) to rats for 12 days. Using the method of binding surface analysis, we determined the Kd and Bmax of PCP receptors. Chronic treatment with haloperidol increased the Kd from 5.6 nM to 7.1 nM, and increased the Bmax from 735 to 1107 fmol/mg protein. These results suggest that PCP receptors may in part be regulated by dopaminergic mechanisms, and that treatment of PCP psychosis may actually prolong clinical symptoms by increasing the density of PCP receptors and therefore increase the exposure of receptors to PCP, which is excreted slowly from the body.

In another study, we examined the effect of chronic opiate abuse on psychotomimetic binding sites of human frontal cortex. Both PCP and σ binding sites were measured. For PCP receptors, the Kd of controls (15.3 nM) and Bmax values of controls (487 fmol/mg protein) were not significantly different from membranes prepared from addicted subjects. For σ binding sites labeled with [3 H]DTG, there were also no significant differences between the two groups. The failure of these receptor systems to demonstrate any significant changes as a result of chronic opiate abuse suggests that these receptor systems are not involved in the process of tolerance and dependence. Alternatively, psychotomimetic binding sites may have been altered in brain areas not sampled in this study.

Some unnatural opiates, which do not interact with classical opiate receptors, interact with PCP receptors. Among their many pharmacological actions, drugs that bind to the PCP receptor antagonize the actions of glutamic acid mediated via the NMDA excitatory amino acid receptor, which leads to their potential use as anti-ischemic and anticonvulsant agents. Despite an enormous effort, researchers have not yet been able to identify a PCP receptor antagonist,

which would be useful for research and therapeutics. Chemical modification of unnatural opiates as a means to produce a PCP antagonist or PCP agonists with properties different than PCP, has not been fully explored. Towards this end, we determined the equilibrium dissociation constants of eight enantiomeric pairs of opiates for the rat brain PCP receptor, and identified (+)pentazocine as a ligand potentially suitable for imaging σ binding sites using PET scans.

Significance to Biomedical Research and the Program of the Institute

1. Progress in pharmacology proceeds as new tools become available. Our ongoing collaboration with Dr. Rice continues to be fruitful, and promises to yield small molecules important to diverse areas of neuroscience.
2. Our work in the area of opiate receptors has led to an increasingly precise definition of the various subtypes of receptors, possible mechanisms by which certain endogenous opioids modulate the effects of others, new insights into the mechanisms of tolerance and dependence, and the development of new tools with which to study opiate receptor mechanisms. The endogenous opioid peptides and their receptors play an important role in the regulation of mood, and a variety of physiological functions, including the immune function. Opiates are major drugs of abuse, representing a significant public health problem that includes a high rate of infection with HIV in that population. Our preclinical studies in the area of opiate pharmacology promises to contribute toward solving this public health problem.
3. Stroke and seizure disorders are indirectly, and perhaps directly in some cases, implicated in a variety of psychiatric disorders. Our preclinical work in the area of PCP receptors relates directly to the development of new anti-ischemic and anticonvulsant agents, and promises to provide new understanding of the mechanisms of psychotomimesis.
4. Our preclinical research on psychotomimetic binding sites may lead to new understanding of psychotic disorders, such as schizophrenia, which is a debilitating psychiatric disorder targeted for research by the NIMH.

Proposed Course

Project 1. Characterization of CNS Opioid Receptors

1A. Development of small molecules as research tools. Our collaborative work with Dr. Rice will continue with efforts to identify irreversible ligands for opioid κ receptors. We will use these agents to explore the possible existence of multiple subtypes of κ receptors and will also use opioid κ receptors as *in vivo* "antagonists" to determine which biological effects of endogenous opioid might be mediated by these receptors.

We will continue to identify more potent δ -selective irreversibles by examining the activities of the four enantiomers of superFIT. The goal of our studies is to develop selective δ antagonists for use in pharmacological studies.

1B. Studies of Opioid Receptor Subtypes

κ receptors. Work in progress suggests that guinea pig brain possesses four subtypes of κ receptors. We will fully characterize these four putative subtypes and conduct comparative studies with rat and human brain. Correlative autoradiographic studies will be carried out in collaboration with Drs. Herkenham and Brady (Unit on Functional Neuroanatomy, NIMH).

μ receptors. The existence of μ receptor subtypes will be explored further using several methods, including chronic drug treatments. In this study, we hope to demonstrate selective upregulation/down regulation of the putative subtypes. The physiological roles of the μ receptor subtypes will also be explored.

Opiate receptor complex. Using site-directed acylating agents, we will generate membrane preparations that possess only the receptor complex. The properties of this complex will then be studied in detail, using [3 H]cycloFOXY to label the μ binding site, and [3 H]DADL to label the δ binding site of the receptor complex. Taking advantage of the fact that [3 H]cycloFOXY is a high yield photoaffinity ligand, we will collaborate with Dr. Wayne Bowen (Brown University) in isolating and characterizing the receptor complex.

In collaboration with Dr. Holaday (Walter Reed Army Institute of Research), we will continue characterizing the functions of the receptor complex *in vivo*. These studies will focus on the opioid receptor subtype altered by the irreversible μ antagonist, β -funaltrexamine. If results show that β -FNA selectively alkylates the receptor complex, then antibodies raised against β -FNA will be used by Dr. Bowen in biochemical studies. In collaboration with Dr. Porecca (University of Arizona), we will continue exploring the ability of subanalgesic doses of opioid peptides to either antagonize or potentiate μ -mediated antinociception, which are events thought to be mediated via the receptor complex.

1C. Mechanisms of morphine tolerance and dependence. Further work in this area will develop in several directions. We will explore the hypothesis that chronic morphine and chronic naltrexone upregulate different populations of μ binding sites, and that only one of the upregulated binding sites are sensitive to β -FNA. We will explore the role of serotonergic systems in the observed upregulation.

Overall, we wish to explore the question: why does the chronic administration of opiate antagonists upregulate opioid receptors when these systems are tonically inactive? Perhaps the level of tonic activity is too low to be detected by acute challenge with an antagonist, but is detectable as an upregulation with chronic challenge. Alternatively, the finding that chronic administration of both agonist and antagonist upregulate opioid binding sites suggests a more complex explanation.

It is possible that the chronic administration of morphine and naltrexone releases endogenous peptides, such as cholecystokinin-8, α -MSH, dynorphin, β -endorphin $_{1-27}$, endogenous phe-met-arg-phe-NH $_2$ -like peptides, and met 5 -enkephalin, which have been shown to antagonize opioid-mediated responses alone or combined with these "anti-opiates" will be shown to participate in the development of tolerance, dependence, and the observed upregulation.

This model potentially provides a mechanism to explain the concurrent development of tolerance and dependence, which is the increasing sensitivity of the organism to challenge with an antagonist as tolerance develops. Continued administration of morphine causes increasing release of anti-opiates into the cerebrospinal fluid, which distributes throughout the CNS, upregulating the opiate receptor complex, attenuating the effects of morphine, and thereby producing tolerance. The administration of exogenous antagonist (i.e. naloxone) synergizes with endogenous anti-opiates to produce the withdrawal syndrome. The more tolerant the animal, the more anti-opiate is released, which results in a physiological context where less naloxone is required to precipitate withdrawal.

This speculative but testable model provides a potentially integrated role for the anti-opiate peptides, a potential mechanism for the development of tolerance and dependence, and an explanation for why chronic administration of agonist and antagonist upregulate receptors. This model is also consistent with growing evidence for parasynaptic transmission via the CSF as a major conduit of information flow in the CNS (Herkenham, 1987). Thus, we plan to explore this novel hypothesis of tolerance and dependence using a variety of experimental approaches.

Project 2. Studies of CNS Psychotomimetic Binding Sites

In collaboration with Dr. Rice, we will continue to develop selective acylators of PCP and σ receptors and will begin to develop a binding assay for the recently described binding site for nonclassical cannabinoids. This assay will be used to screen for putative irreversible ligands and possible ligands suitable for PET studies.

In the area of serotonin receptors, we will use custom-synthesized ^{125}I -base radioligands and their ^{127}I -analogs to characterize serotonin receptor subtypes. Efforts toward synthesizing subtype selective irreversible agents will begin with initial agents targeted toward the 5-HT $_1\text{A}$ and 5-HT $_1\text{B}$ receptor subtypes.

PCP, its analogs, certain opiates, and MK801, act as noncompetitive antagonists at the NMDA receptor complex, producing anticonvulsant and anti-ischemic activities *in vivo*. Regarding work in progress on PCP receptors, using guinea pig membranes, we have suggested the existence of two classes of PCP binding sites, one associated with the NMDA receptor (site 1), and the other not (site 2). We plan to explore this hypothesis further, since an obvious implication of this hypothesis is that the psychotomimetic effects of PCP might be mediated via site 2, while potential therapeutic effects might be mediated via site 1. The implications of this hypothesis for drug design is also obvious, since successful therapeutic agents would be those highly selective for site 1. Various compounds synthesized in Dr. Rice's laboratory will be screened with this in mind.

Similarly, preliminary data suggest the existence of two classes of σ binding sites, and work on this project will continue, including autoradiographic visualization of the two sites and detailed structure activity studies. In addition to the intrinsic interest of this work, ligands that interact at PCP sites also interact with σ binding sites. Thus, a detailed working knowledge of σ receptors is required for development of compounds selective for classes of PCP receptors.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00433-08 LCS

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Neuropeptides and Biogenic Amines in Neuroendocrine Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.M. Saavedra, Chief, Unit on Preclinical Neuropharmacology/LCS/NIMH
 Others: E. Castren VF/LCS/NIMH F.M.A. Correa Guggenheim Fellow
 J. Laitinen VF/LCS/NIMH J.S. Gutkind Int. Fogarty Fellow
 A. Himeno VF/LCS/NIMH D. McKenna PRAT Fellow/NIGMS
 J.E.B. Pinto VS/LCS/NIMH A.J. Nazarali Alberta Found. Fellow
 T. Torda VS/LCS/NIMH S. Guilhaume Guest Researcher
 C. Gonzalez VF/NI/NHLBI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

5.5

PROFESSIONAL:

5.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Receptors for the psychotomimetic dimethoxyphenylisopropylamine (DOI) were identified in discrete brain areas and shown to cross-displace with LSD. These receptors were shown to be of the 5HT₂ type. Chronic DOI administration to rats resulted in a down regulation of cortical 5HT₂ receptors. New very sensitive quantitative radioimmunocytochemical methods were developed. These methods allowed for the localization and quantification of many antigens in individual rat brain nuclei in tissue sections, by autoradiography and quantitative microdensitometry and have a 100-fold higher sensitivity than classical RIA. Quantitative autoradiography was utilized for the study of amine and neuropeptide receptors in human lymphocytes and platelets, with a ten-fold increase in sensitivity over previous assays. We have found profound changes in paraventricular nucleus angiotensin II receptors in conditions of altered CRF secretion in rats, suggesting a role for central and peripheral angiotensin in CRF release and in stress. We have described and characterized angiotensin II and atrial natriuretic peptide receptors in sympathetic ganglia of the rat and found alterations in these receptors in conditions of altered sympathetic activity, suggesting a role for these peripheral/local hormones in the regulation of autonomic function. We applied quantitative autoradiographic techniques to study the classification of beta-adrenoceptors in discrete brain areas and parts of the conduction system of the heart.

PROJECT DESCRIPTION

Objectives

To study the role of central and peripheral biogenic amines and neuropeptides in the control of the pituitary function and specifically during stress, their role in the regulation of the autonomic nervous system, the cardiovascular system, and their control of fluid metabolism.

To develop new quantitative autoradiographic methods for the determination of the content of many antigens, including neuropeptides, in discrete brain nuclei from thin tissue sections.

To develop sensitive, quantitative methods for the study and characterization of biogenic amine and neuropeptide receptors in man, with emphasis in the application of quantitative autoradiography to the study of receptors in human circulating cells.

To clarify the relationship between brain receptors for different psychotoxic compounds, and to identify the principal brain areas where interactions between different receptors occur. To study the role of specific dopamine D₁ and D₂ receptors in the brain, and to clarify the effects of classical and atypical neuroleptics in these receptors.

Methods Employed

Neuroanatomical, surgical, biochemical (RIA, gel electrophoresis, radioenzymatic assays) and autoradiography with image analysis combined with computerized microdensitometry.

Major Findings

1. As determined by autoradiography, the iodinated 5HT₂ agonist with psychotomimetic properties, (4-iodo-2,5-dimethoxy-phenylisopropylamine) (¹²⁵I-DOI) binds specifically to rat cortex and claustrum. This binding is displaced not only by unlabeled DOI but by unlabeled LSD. ¹²⁵I-LSD binding is selectively displaced by unlabeled DOI in these structures, indicating a cross displacement in specific brain areas by two different kinds of psychotomimetic compounds.

2. Chronic administration of unlabeled DOI to rats results in a down regulation of 5HT₂ receptors in cortical membranes, as determined by ³H-ketanserin binding.

3. A series of new methods, utilizing Protein A, were developed for immunocytochemistry and for radioimmunocytochemistry and quantitative autoradiography. The utilization of Protein A for immunocytochemical methods simplifies the classical PAP techniques, since there is no longer need for the use of a second antibody. The use of ¹²⁵I-protein A has the additional advantage of the combination with autoradiography to yield permanent records of increased sensitivity. The comparison with ¹²⁵I-standards allows the

quantification of antigens in single nuclei of thin brain sections, with a 100-fold increase in sensitivity over classical RIA techniques. The methods have been validated for tyrosine hydroxylase, methionine enkephaline, angiotensin converting enzyme and vasopressin, and they have the potential of application to many other tissue antigens.

4. We have characterized and classified β -adrenoceptors in single rat peripheral sympathetic ganglia, including the stellate ganglia. Most of the receptors are of the β_2 -type. This, and the finding of a large proportion of β_2 adrenoceptors in the heart specialized conduction system, suggest a possible role for selective β_2 blockers in alterations of heart rhythm and in anxiety states with a substantial cardiac component.

5. A large proportion of β_2 adrenoceptors was identified in both rat and human heart valves (mitral and aortic), suggesting a role for locally formed or circulating epinephrine in pathological states involving the valve leaflets.

6. Specific alterations of angiotensin II and atrial natriuretic peptide receptors in selective areas of the rat brain occurred in animals treated chronically with the inhibitor of angiotensin II synthesis, enalapril. The response after enalapril treatment was different for normotensive and for spontaneously (genetic) hypertensive rats. These results indicate that: a) both central angiotensin II and atrial natriuretic peptide receptors are involved in the regulation of blood pressure; b) the systems are regulated differently in genetic hypertension; c) angiotensin converting enzyme inhibitors have actions in the brain and d) these actions are different in normotensive and hypertensive animals.

7. Melatonin, vasoactive intestinal peptide, and neuropeptide Y receptors have been identified and characterized in selective brain areas, retina, and peripheral tissues of the rat by quantitative autoradiography.

Significance to Biomedical Research and to the Institute

The application of quantitative autoradiography to human samples will open new possibilities for clinical research. These techniques are 10-fold more sensitive than classical membrane binding methods, and allow the simultaneous determination of multiple receptors in circulating cells obtained from a small (20 ml) blood sample.

The findings of cross-displacement between different psychotomimetic drugs, specifically located in selective brain areas may help in the understanding of the pathophysiology of hallucinations and the mode of action of psychotomimetics.

In addition to receptors and enzymes, quantitative autoradiographic methods now allow for the quantification of antigen (neuropeptides) concentrations in single rat brain nuclei. These techniques will be useful to study the metabolism of neuropeptides in selected brain areas in greater detail.

Two peripherally formed hormones, angiotensin II and atrial natriuretic peptide, have actions in the brain and may play important roles during stress. These findings could help to further understand the biochemical basis of stress.

Proposed Course

We plan to further explore the interaction between receptors for different classes of psychotomimetic compounds in selective brain areas, the interaction of these receptors and dopamine receptors after acute and chronic administration of psychotomimetic compounds to rats, and the effect of classical and atypical neuroleptics. We hope to reach a further understanding about the sites of action of psychotomimetic compounds, and about the biochemical basis for the therapeutic effect of neuroleptics.

The newly developed autoradiographic methods will be available to clinical investigators for collaborative studies on the role of biogenic amine and neuropeptide receptors in affective illnesses and schizophrenia.

We plan to continue experiments in stress to further clarify the role of peripherally formed hormones and central peptidergic systems in the regulation of the formation and secretion of CRF and in the stress reaction.

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In Press

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00447-19 LCS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Amine Neurotransmitters and Metabolites in Mental Illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

William Z. Potter, M.D., Ph.D. Chief, Section on Clinical Pharmacology LCS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Work on this project has been transferred to project Z01 MH 01350-11 LCS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01850-11 LCS

PERIOD COVERED

October 1, 1987, through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Pharmacology of Antidepressants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

William Z. Potter, M.D., Ph.D., Chief, Section on Clinical
Pharmacology, Laboratory of Clinical Science, NIMH

COOPERATING UNITS (if any)

Clinical Psychobiology Branch; Clinical Neuroscience Branch; and Laboratory
of Clinical Studies, NIAAA

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

5.4

PROFESSIONAL:

3.6

OTHER:

1.8

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- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies to explore the mechanism of action of antidepressants in patients and volunteers have expanded to incorporate new analytical and biochemical approaches. Highlights of the last year include:

1. New algorithms for relating neurotransmitter measures allow us to distinguish both depressed and schizophrenic patients from controls as well as treatment responders from nonresponders. These findings provide our first direct evidence that measures of neurotransmitter interactions in patients may be used to better understand drug action.
2. We find in volunteers that chronic lithium "up-regulates" components distal to adrenergic receptors on platelets and lymphocytes without affecting other indices of nonadrenergic function, such as release or turnover. This provides direct support for a primary effect of lithium on signal transduction and suggests that its inhibitory influence is compensated in vivo.
3. Dose response studies on intravenous administration of the atypical antidepressant alprazolam have established the appropriate dose for a pharmacological challenge in man potentially reducing ACTH and cortisol, stimulating GH and suppressing norepinephrine in plasma.
4. The selective alpha-2 antagonist, idazoxan, appears to be an effective antidepressant and is able to produce sustained increases in plasma norepinephrine without altering cardiovascular parameters. These findings necessitate a reinterpretation of previous studies on catecholamines.

Other Professional Personnel:

Matthew Rudorfer	Senior Staff Fellow	LCS/NIMH
John Hsiao	Medical Staff Fellow	LCS/NIMH
Husseini Manji	Medical Staff Fellow	LCS/NIMH
Emile Risby	Guest Researcher (NRSA)	LCS/NIMH
Ivan Mefford	Special Expert	LCS/NIMH
Laura Fochtmann	PRAT Fellow	LCS/NIMH
Jose Bitran	Visiting Fellow	LCS/NIMH
Markku Linnoila	Chief	LCS/NIAAA
Dennis Murphy	Chief	LCS/NIMH
Thomas A. Wehr	Chief	OP/NIMH
David Pickar	Chief, Section on Clinical Studies	NSB/NIMH
Judith Rapoport	Chief, Child Psychiatry Branch	CHP/NIMH
Herbert Weingartner		LCS/NIMH

Project Description:

Our central aim is to understand the effects of major somatic antidepressant treatments on the monoamine neurotransmitters and their signal transduction in man. Systematic studies of drug action in normal volunteer controls and depressed patients controlling for pharmacokinetic and pre-drug physiologic variance have permitted demonstration of both predicted and unexpected biochemical alterations following treatment with drugs or convulsive therapy having widely differing acute primary effects.

Comparison of biochemical effects in CSF, plasma and urine in the same patients continues with efficient high performance liquid chromatography assays and, when coupled with physiologic, behavioral and neuroendocrine measures, allows for clearer systems interpretations of changes.

Moreover, peripheral neurotransmitter receptors on formed elements in blood are studied. State-of-the-art measures of norepinephrine (NE), serotonin (5HT), dopamine (DA), and their metabolites are made under controlled conditions both cross-sectionally in time and longitudinally in order to identify interrelationships, to test assumptions about the regulation of these neurotransmitter systems, and therefore to definitively describe effects of antidepressants as they relate to these neurotransmitter systems.

Methods:

The neurotransmitter systems of patients with either unipolar or bipolar major affective disorder are characterized after at least a 3-week drug-free period and then between the 3rd and 5th week following treatment. Certain parameters, such as concentrations of neurotransmitters and metabolites in blood, urine and cerebrospinal fluid, are studied in all subjects. Patients admitted at steady-state of an antidepressant drug are also studied serially during the withdrawal phase. Parallel studies are performed in healthy volunteers and animal models when feasible, as described below.

Treatments are chosen as prototypes to produce maximal effects on selected target biochemical systems, such as inhibition of NE uptake after desipramine or of MAO type A after clorgyline. In cases (see below) where there is no well-defined biochemical target established (e.g. lithium), established "therapeutic" protocols are followed. Novel putative anti-depressants with no clear biochemical specificity, such as alprazolam, are studied in terms of their biochemical effects after acute and chronic dosing and, in the case of ECT, serially (generally weekly) throughout the course of treatment.

Studies in college-age volunteers housed on the unit are of shorter duration (up to two weeks of active drug) and may include traditional drugs such as lithium, as well as new drugs such as the α_2 antagonist, idazoxan.

Specialized pharmacokinetic and baseline biochemical studies are performed in volunteers age- and sex-matched to our accumulated patient population. These volunteers come to the clinic on the day of the study or are admitted for an overnight accommodation to the research unit.

Analysis of NE, 5HT, DA and their metabolites is carried out as described in a separate report, Z01 MH 01855-04 LCS. Adrenergic receptor function is measured in platelets with a focus on NE and EPI inhibition of PGE_1 stimulated cyclase and in lymphocytes with a focus on agonist displacement of ICYP binding.

Additional methods are utilized for preclinical experiments. These include use of chemically differentiated HL60 cells to explore the effects of lithium on signal transduction observed in clinical studies. Stimulated intracellular pH changes, calcium mobilization and PI turnover are measured before and after "chronic" exposure to lithium. In a totally different approach to modeling human studies, microdialysis of CSF in rats is being developed to see if parallel neurotransmitter changes occur after treatments such as ECT which we have found to have specific effects in humans.

Findings to Date:

1. The algorithms which we have developed over the last three years for studying neurotransmitter interactions in humans have been prospectively applied to both diagnostic and treatment-related questions. Two types of new findings have emerged:

- a) In collaboration with Dr. David Pickar of the NSB and Dr. Hans Ågren of the University of Uppsala, we find that the ratio of HVA/5HTAA in CSF, a possible measure of dopaminergic-serotonergic interactions, is reduced in both depressed and schizophrenic patients as compared to controls. There is a substantial proportion of schizophrenic patients whose values for the HVA/5HTAA fall more than two standard deviations below the mean value in controls.

- b) The normal robust intercorrelation of MHPG, 5HIAA and HVA which we have previously found in controls and depressed patients who respond to treatment is dramatically reduced in unmedicated and decompensated schizophrenic patients. Treatment with neuroleptics "normalizes" the intercorrelations; whether this change occurs as a function of degree of response is not yet known.

2. We completed a study on selected biochemical parameters following two weeks of lithium to volunteers. Our preliminary results that lithium had no measurable effect on most indices of NE output was confirmed. We did observe increased total excretion of NE in the urine (with no changes in plasma), suggesting a direct effect on the kidney.

Our most interesting finding was that lithium had differential effects on signal transduction in platelets and lymphocytes. In preparations from blood drawn 14 hours after the last dose of lithium, basal adenylate cyclase was increased in platelets and reduced in lymphocytes. Gpp(NH)p stimulation was increased in both platelets and lymphocytes. In the latter there were no changes in the E_{max} or affinity of beta receptors. Lithium, however, altered the agonist displacement of ICYP binding from lymphocyte beta receptors such that the ratio of dissociation constants of the low-to-high affinity states was increased. Taken together, these results suggest a form of up-regulation such that following chronic treatment beta receptors "compensate" for the known ability of lithium to inhibit agonist-induced adenylate cyclase activity when added to in vitro preparations.

3. In complementary preclinical experiments and in collaboration with Dr. Martin Zatz, we have tested the ability of lithium in vitro to alter signal transduction through an amiloride sensitive site in neutrophils, i.e. FMLP-stimulated superoxide formation and/or intracellular pH. We find no acute effect of lithium but, using HL60 cells which have been pre-exposed to the drug, we show a "selective" alteration in the FMLP-induced pH changes; i.e. in the absence of effects on PI turnover or intracellular calcium mobilization.

4. Following an initial study of the acute biochemical effects of intravenous alprazolam, we have done a more detailed dose response study. We find that growth hormone is stimulated at doses which do not affect plasma NE. Furthermore, by working at a lower dose, we can investigate shifts of response both as a function of illness and treatment (see below).

5. A few patients have been entered on a trial of the selective α_2 antagonist, idazoxan, with a striking initial finding that there is a sustained doubling of plasma NE in the presence of unchanged cardiovascular parameters. As expected, idazoxan blocks the growth hormone response to alprazolam but surprisingly "destabilizes" the NE response. These findings demonstrate that chronic α_2 blockade produces a new and partly "compensated" set point of the noradrenergic system; the consequence of this for psychiatric states is being explored.

2. Preclinical studies utilizing cultured cells on the mechanism of action of lithium will focus on the possibility of effects on protein kinase C, leading to altered phosphorylation of membrane components involved in the mediation of FMLP-induced pH changes and other types of signal transduction.
3. Free normetanephrine (NM) in CSF will be explored in depressed patients before and after treatment with ECT and idazoxan; we predict no effect of the former and an increase after the latter. If our predictions hold, we will proceed to develop an algorithm involving NM as well as NE and MHPG to better describe the "state" of the noradrenergic system in humans.
4. Again with regard to ECT and idazoxan, which have been suggested to affect cognitive function independent of clinical response, in collaboration with Dr. Herbert Weingartner we plan to carry out longitudinal studies of the treatments on a variety of mental functions known to be selectively altered in various pathologic conditions. A protocol for chronic (2 week) administration of idazoxan to normal volunteers is being developed.
5. In order to better understand the mechanism by which treatments alter plasma amines, we are exploring the possibility that neuroleptics, lithium and ECT have effects on the renal clearance of HVA, NE and MHPG. Recently developed HPLC assays for unconjugated amines and their metabolites in urine make this study possible.
6. PET studies with 2DG of patients before and after treatment with ECT have been initiated. Because of our earlier findings of increased D₁ receptors after ECT in rats, we will look for opportunities to carry out either PET or SPECT studies in the same paradigm with a D₁ antagonist. And, based on our promising results with idazoxan, we are exploring the possibility of utilizing a much more selective alpha₂ antagonist, atipamezole, as a ligand to be used in brain imaging.

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In Press

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01855-04 LCS

PERIOD COVERED

October 1, 1987, through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Central Neurochemistry Service

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

William Z. Potter, M.D., Ph.D., Chief, Section on Clinical
Pharmacology, Laboratory of Clinical Science, NIMH
Ivan N. Mefford, Ph.D. Special Expert/LCS/NIMH
Sanford P. Markey, M.D. Chief/AB/LCS/NIMH

COOPERATING UNITS (If any)

Section on Analytical Biochemistry and Section on Biomedical
Psychiatry, LCS, NIH; Laboratory of Clinical Studies, NIAAA

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.2

PROFESSIONAL:

0.7

OTHER:

3.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The central neurochemistry service has continued a high level of activity in analytical methods development and analysis of human fluids and tissues. Routine analysis is presently available for MHPG, HVA, 5HTAA, 5-HT, NE and normetanephrine in plasma. These compounds as well as VMA, DOPAC, epinephrine, dopamine, metanephrine, and 3-methoxytyramine can be measured in urine. In addition, CSF measures of epinephrine, NE, DA, HVA, 5HTAA, MHPG, free and conjugated metanephrine and normetanephrine, and 5-HT are possible. New methods developed include a reverse phase HPLC procedure for separation of stable isotopes of dopamine. This will allow metabolic studies of dopamine in humans and animals without the use of GC-MS or radioactive ligands. A new assay for 5-HT in CSF was developed with limits of detection <10 pg/ml. This method takes advantage of microbore HPLC technology and a "noneluting matrix" approach to achieve the low detection limits. After studying more than 100 human samples, mean and median concentrations were found to be near the limit of detection, suggesting that CSF 5-HT is unlikely to provide insight into brain 5-HT neuronal activity. A new GC-MS assay for metanephrine and normetanephrine, the O-methylated metabolites of epinephrine and norepinephrine, respectively, has been developed. Conjugated and free forms of these compounds can be determined in CSF, allowing for greatly improved clinical studies on the action of MAOIs in humans.

Project Description:

The Central Neurochemistry Service provides a centralized analytical facility whose focus is the measurement of neurotransmitters and metabolites in physiological fluids generated by the clinical intramural research effort. Four technicians are presently provided by NIMH and one by NIAAA. These individuals perform routine assays and participate in development of new assays; four of these use primarily HPLC while one performs assays and methods development on GC-MS in collaboration with the Section on Analytical Biochemistry (Dr. Markey).

Methods:

As noted above, the major analytical effort involves high performance liquid chromatography (HPLC) with electrochemical detection. Using recently developed reagents for separation of biogenic amines and microbore technology, selective detection of catecholamines at the 45 pg/ml level has been accomplished. Novel ion-pairing reagents allow "on column" concentration of samples (amines) eliminating tedious derivatization and extraction steps.

1. Amperometric detection when coupled to microbore HPLC offers significant (~50 fold) signal enhancement when compared to conventional HPLC or to coulometric detection.
2. Using a "non-eluting matrix" approach, biogenic amines can be selectively concentrated "on column" eliminating the necessity of derivatization and extraction.
3. HVA, 5HIAA and MHPG can be determined simultaneously in a single plasma extract. Separate assays for plasma 5HIAA and HVA were combined with the MHPG assay, eliminating separate sample preparation steps and analyses.
4. Columns can be prepared "in house" via slurry packing, which is a considerable savings in time and cost. This is now offered as a service to other NIMH laboratories.

Findings:

1. Using amperometric detection, we are able to quantitate concentrations of epinephrine and serotonin in the 100 femtomole/ml range in cerebrospinal fluid. These methods (a combination of 1 and 2) are now routinely applied to CSF samples. Epinephrine concentrations are routinely found to be 1-5 pg/ml while serotonin is usually found in the 10-100 pg/ml range.
2. Reverse phase HPLC is able to separate stable isotopes of dopamine. This is an important development since it will permit investigators to design and carry out metabolic studies of dopamine in humans and animals without resorting to GC-MS.

3. In collaboration with the Section of the Chief, LCS, a new assay for serotonin in CSF with detection limits below 10 pg/ml was developed utilizing microbore HPLC with a non-eluting matrix approved for on-column concentration of up to 100 microliters of CSF. Over 100 human samples have been studied, revealing mean concentrations below or at the level of detection -- a finding which calls into question results from most previous studies and suggests that the higher values reported in the literature result from platelet contamination or some other error. Our results further suggest that measures of 5HT in CSF are unlikely to be of general value in clinical research.

4. A new GC-MS assay for metanephrine and normetanephrine, the O-methylated metabolites of epinephrine and norepinephrine, respectively, has been developed. Free and total (conjugated plus free) can be determined in CSF allowing for greatly improved clinical studies on the action of MAOIs in humans (see below).

Significance to Biomedical Research and to the Program of the Institute:

Neurotransmitter system function is implicated in major psychiatric illness, in behavioral medicine (e.g. responses to psychological and physiological stress) and in the mode of action of psychoactive as well as cardiovascular drugs. Improved methods for studying these neurotransmitter systems are crucial to understanding their operation in humans since adequate animal models or in vitro systems do not exist.

Only by fully and accurately quantitating neurotransmitters and their metabolites will it be possible to distinguish alterations of output vs those of metabolism and to relate amount to function. These techniques provide the best current hope of biochemically identifying individuals with psychiatric disease, at risk for such illness and/or most likely to respond to specific treatment.

Proposed Course:

With full-time professional direction of the laboratory, we plan to achieve the following over the next year:

1. Continue to assess the usefulness of plasma MHPG vs plasma norepinephrine and normetanephrine as an index of noradrenergic function.
2. Measure free amines and metabolites in plasma and urine to study the renal clearance of these compounds.
3. Assess the functional role of epinephrine formation in brain via CSF measurements in psychiatric populations and following drug intervention.
4. Study mechanisms involved in metabolism and clearance of norepinephrine in the periphery by studying free and conjugated derivatives in plasma and urine.

Papers for Which the Central Lab Provided Analytical Support

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In Press

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01860-02 LCS

PERIOD COVERED

October 1, 1987, through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Epinephrine in Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Ivan N. Mefford, Ph.D.
Monica I. Masana, Ph.D.Special Expert, LCS/NIMH
Visiting Fellow/LCS/NIMH

COOPERATING UNITS (if any)

Section on Analytical Biochemistry and Section on Biomedical
Psychiatry, LCS, NIH; Laboratory of Clinical Studies, NIAAA

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

1.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

While epinephrine is the least concentrated of the catecholamines in mammalian brain, our view has developed that it has a significant role in regulation of sensory activation. This is thought to be due to its formation in extragranular sites, both intra and extraneuronally due to the compartmentalization of PNMT (phenylethanolamine N-methyltransferase). This view has prompted the study of the sites and cell types in which epinephrine may be synthesized. We have observed synthetic capacity in intrinsic neurons of the hypothalamus, incapable of storage of epinephrine as well as in tanycytes, the ependymal lining of the third ventricle. We have demonstrated that PNMT activity in the medulla is modified by stress while hypothalamic PNMT is relatively unaffected. Further, PNMT activity does not appear to be tightly linked to epinephrine content, suggesting that PNMT-containing neurons in the medulla are a subset of noradrenergic neurons. Inhibition of PNMT leads to motor activation and sensitivity to auditory cues in young rats, supporting the hypothesis of an inhibitory role for epinephrine in regulation of sensory activation.

Project Description:

The goal of this work is to understand and describe the metabolism and physiological of endogenously formed epinephrine in the mammalian brain. This project is being studied using several approaches.

Metabolism of epinephrine. Accumulated pharmacological data suggest that enzymatic formation in the hypothalamus is dissociated from the storage sites of epinephrine. Evidence suggests that this synthesis may occur in non-neuronal elements. We are examining the possibility that this synthesis occurs in glial cells, specifically astrocytes. This is being studied in both astrocyte cultures and astrocytes isolated from adult rat brain. Future studies will examine the properties of uptake of norepinephrine into these cells and characterization of this N-methyltransferase.

Pharmacological manipulation of epinephrine synthesis. Considering epinephrine as an extraneuronal metabolite of norepinephrine suggests that the physiologically relevant pool of epinephrine is found in extracellular space. This pool is actively modified by the release of norepinephrine from selected neuronal populations, particularly the projections of the A_1/C_1 and A_2/C_2 cell body groups. Epinephrine synthesis via PNMT should provide slow elevation of this pool but a rather short time course for clearance following enzyme inhibition. Numerous pharmacological manipulations of noradrenergic release and reuptake and metabolism can be studied including MAO inhibitors, uptake inhibitors, α_2 receptor agonists and antagonists and inhibitors of epinephrine synthesis. The effects of these manipulations can be measured in extracellular fluid using equilibrium dialysis. Further, the behavioral consequences of PNMT inhibition can be measured rapidly.

Methods:

Equilibrium dialysis. Dialysis probes are prepared to provide optimum recovery and regional selectivity. Dialysis tubing, 250 μ m in diameter, is used to prepare probes as described by Zetterstrom & Sharp and Ungerstedt. Collected dialysates are analyzed for amines using microbore HPLC with amperometric detection. Further selectivity is obtained by using IGEPON T-77 as a chromatographic modifier.

All other tissue, plasma and/or CSF analyses are accomplished using published HPLC techniques.

Findings:

1. A subset of hypothalamic neurons contains PNMT but does not store epinephrine.
2. Radial glial cells of the ependymal lining of the third ventricle (tanycytes) contain PNMT and do appear to contribute to the normal biosynthetic and storage pool of hypothalamic epinephrine.
3. Astrocytes cultured from foetal rat hypothalamus have epinephrine-synthesizing capacity.

4. PNMT inhibition provides marked prophylaxis against ethanol or barbiturate intoxication, but not anesthesia.

5. PNMT inhibition is markedly activating in young rats (days 18 and 19) but inactivating in later life (day 25 adulthood).

Significance:

Understanding the metabolism and functional significance of epinephrine in mammalian brain may provide a great deal of insight into the mechanism of action of several classes of drugs. If, as this research proposes, epinephrine is an extraneuronal metabolite of norepinephrine, any drug affecting norepinephrine release, reuptake storage and metabolism would affect the hormonal pool of epinephrine. It is proposed that one of the functions of epinephrine in brain is tonic regulation of the level of arousal and reactivity to sensory stimuli. Some evidence suggests that epinephrine synthesis is important in reward mechanisms. Consequently, epinephrine synthesis may be important in antidepressant efficacy. Our work, already completed, suggests an important role for epinephrine in intoxication and tolerance to sedative hypnotics. Further, epinephrine synthesis may be related to the mechanism of action of drugs used to treat hyperactivity.

Proposed Course:

Test the hypothesis that hypothalamic epinephrine is both an extraneuronal and intraneuronal metabolite of norepinephrine, in hypothalamus and medulla, involved in regulation of arousal.

A) Assessing the actions of intoxicants, anesthetics and sedative hypnotics in awake, unanesthetized animals on extracellular epinephrine, norepinephrine, dopamine and serotonin.

B) Assessing effects of classical adrenergic drugs, amphetamine, cocaine, tricyclics, neuroleptics and MAO inhibitors, on extracellular epinephrine.

C) Studying synthesis of epinephrine and uptake of norepinephrine in non-neuronal brain cells.

D) Studying the behavioral consequences of PNMT inhibition.

Publications

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In Press

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00787-09 LCS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Mechanisms of Isolation Call in Squirrel Monkey (*Saimiri sciureus*)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.D. MacLean, M.D. Section on Comparative Studies of Brain and Behavior LCS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00796-03 LCS

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cytochemical Tracing of Thalamic Connections with Midline Frontal Cortex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. D. MacLean Intramural Research Scientist LCS, NIMH
Other:

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

0.9

PROFESSIONAL:

0.3

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This anatomical study stems from a preceding project (Z01 MH 00787-08 LCS), in which it was shown in squirrel monkeys that a strip of midline frontal limbic cortex is involved in the spontaneous production of the separation call. The call ranks as a basic mammalian vocalization, serving originally to maintain maternal-offspring contact. The purpose of the present project is to define the respective connections of the frontal midline limbic and neocortex with the thalamus and other parts of the cerebrum. The two preceding reports dealt with the findings obtained with the use of WGA-HRP (wheat germ agglutinin conjugated to horseradish peroxidase) as a cytochemical tracer. In the present phase of the study fluorescent tracers for revealing double labeling of nerve cells are being used to assess the extent of thalamic innervation of midline areas by collateral nerve fibers. Injection of two different dyes into limbic and neocortical areas between frontal planes F22-F18 of the brain atlas revealed almost no evidence of double labeled cells in the thalamus. Another purpose of the study is to obtain information about cerebellothalamic relationships of the limbic "vocal area" and of the rostral supplementary motor area.

Project Description:

Objectives: In the study on the cortical representation of the separation call in Saimiri monkeys (Z01 MH 00787-08 LCS), it was found that midline frontal ablations failed to induce significant retrograde thalamic degeneration. Long-recognized, comparable findings in macaques led to the assumption that this cortex was either athalamic or that degeneration did not occur because of sustaining collaterals. Since a strip of limbic cortex including paragenual area 24, subcallosal area 25, and caudal area 12 was found to be essential for the spontaneous production of the cry, it was anatomically necessary to obtain a definition of the thalamic afferents of this cortex and of the adjacent midline neocortex. Given the information obtained by an initial survey using wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP), fluorescent tracers are now being used for double labeling in an attempt to clarify questions concerning sustaining collaterals. Another question that needs to be resolved is that regarding a linkup with the cerebellum.

Methods Employed: Since this year's renovation of the laboratory interfered with work on this project, it was fortunate that, thanks to Professor Lennart Heimer of the University of Virginia Medical Center, it was possible to conduct some experiments involving overlapping interests with George Alheid and Jose de Olmos. In this work, fluorescent tracers were used for purposes of double labeling, and there was the opportunity in Saimiri monkeys to compare the results of using fast blue and diamidino yellow with two relatively new tracers rhodamine beads, and fluoro-gold. It is to be noted that diamidino yellow labels only the nucleus. The agents were used in their recommended concentration (with 2% DMSO as a vehicle) and in amounts ranging from 100 to 200 nl. So as to avoid more than one operative procedure, all the fluorescent dyes were selected so as to permit a survival time of 10 days. For the experiments with WGA-HRP, the survival time was 3 days.

Major Findings: First, it may be said in general that one may expect excellent results in Saimiri monkeys when using either fast blue or diamidino yellow as tracers, and the number of labeled cells compares to that found with WGA-HRP. In the case of rhodamine, however, the detection of labeled cells is made difficult because, with the filters used, the autofluorescence of lipofuscin which may be quite widespread in the adult Saimiri brain has the same red color. In our limited experience fluoro-gold seems to be poorly transported in the Saimiri brain.

When fast blue and diamidino yellow are injected into different midline limbic and neocortical areas (between frontal planes F22 and F18), the respective labeling in the thalamus usually involves distinctly separated groups of cells with no evidence of double labeling. But even when labeling involves intermingling of cells in the same nucleus, the occurrence of double labeling is rarely evident.

In regard to the question of cerebellofrontal relationships, it should be stated as background that in the macaque the deep cerebellar nuclei project

to the oral part of the ventral posterolateral nucleus (VPLo); caudal parts of the ventrolateral nucleus; area X of Olszewski; and the central lateral nucleus (Asanuma et al., 1983). If the superior lateral nucleus represents an extension of the central lateral nucleus as has been indicated in the macaque, then the findings with WGA-HRP in Saimiri monkeys would indicate a strong linkup of this nucleus with the limbic "vocal area." There exists evidence that the limbic "vocal area" has reciprocal connections with the neocortex of the supplementary motor area (SMA) which is known clinically to have an influence on vocalization. In the present study examination of frontal sections leaves open the possibility that rostral SMA receives some afferents from cells in caudal parts of VL.

Significance and Biomedical Research and the Program of the Institute: This project relates to the general question of brain mechanisms involved in crying and laughter that in clinical neurology has remained one of the most resistant to explication. The present anatomical investigation is concerned with the demonstration of thalamic and other connections of the medial fronto-limbic area found to be involved in the separation cry of squirrel monkeys. As stated in previous reports, this area has extensive connections with the anterior, medial, and intralaminar thalamic nuclei as well as with parts of the ventral anterior nucleus. This year's findings with fluorescent tracers fails to support suggestions of years ago that the midline frontal cortex is extensively innervated by collateral fibers from the thalamus.

In regard to the important question of cerebellothalamofrontal connections, the above findings suggest two possible means of a linkup. Experiments entailing the examination of horizontal and sagittal sections would be helpful in the attempt to obtain clarification in regard to the question of connections with caudal VL. Combined with a research of the clinical literature, the anatomical findings suggest possibilities for a linkup of frontal and strio-pallidoni-rothalamic mechanisms implicated in both the affect and expression of crying and laughter.

Proposed course: To be concluded upon completion of work in progress.

Publications:

MacLean PD and Newman JD. Role of the midline frontolimbic cortex in production of the isolation call of squirrel monkeys, *Brain Res* 1988;450:111-123.

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MacLean PD. A reinterpretation of memorative functions of the limbic system. In: Goldberg E, ed. Festschrift for Aleksandr Romanovich Luria. New York: The IRBN Press, in press.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 MH 00797-03 LCS

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Attachment

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	T.R. Insel	Staff Physician	LCS, NIMH
	A.E. Johnson	Staff Fellow	LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science, NIMH

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

0.8

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the second year of this project we described regional changes in brain oxytocin receptors associated with the onset of maternal behavior. In this, the third year of this project, we further investigated the role of oxytocin in maternal behavior by studying the regulation of oxytocin gene expression using in situ hybridization. Preliminary results reveal increased oxytocin mRNA in the rostral paraventricular nucleus of the hypothalamus following parturition. Electrolytic lesions of this region altered the initiation but not the maintenance of maternal behavior. The role of gonadal steroids on oxytocin receptor number and oxytocin gene expression is currently under study. We have obtained an oxytocin analogue which is highly selective for the oxytocin receptor and which can be labeled with ¹²⁵I for use with in vitro receptor autoradiography. This long-awaited compound, Peptide VI, is the necessary tool to answer a number of questions about the physiologic regulation of oxytocin receptors.

Project Description:

Objectives: Our experimental studies of attachment behavior have cut two ways. One set of studies has investigated the neural substrates of the rat pup response to separation from mother and littermates, i.e. social isolation. In the previous year, we demonstrated decreased availability of brain benzodiazepine receptors during social isolation. One objective during this past year was to extend this observation by investigating the effects of another anxiety-related neurochemical system, the peptide corticotropin releasing factor (CRF). This set of studies is now described in Z01 MH 02219, Animal Models of Anxiety.

Our second approach to attachment behavior has been the study of parental behavior. Previously we demonstrated an increase in brain oxytocin receptors during parturition or following exogenous estrogen administration to ovariectomized females. Several questions emerged from these initial experiments: Would similar effects appear in males? Does oxytocin content increase in brain? How does the removal of oxytocin cell bodies alter maternal behavior? And, from a technical perspective, how can we improve our technique for selectively measuring oxytocin receptors?

Methods Employed: Oxytocin receptors have been studied using *in vitro* receptor autoradiography. Lesions of oxytocin cell bodies have been produced in the paraventricular nucleus of the hypothalamus using stereotaxic electrolytic techniques. We have monitored oxytocin synthesis at the light microscopic level with *in situ* hybridization using a synthetic oligo-nucleotide probe, validated by Northern blot analysis. Finally, to improve the sensitivity and specificity of the oxytocin receptor binding technique, we have worked with private contractors to synthesize a highly selective oxytocin analogue (Peptide VI) which can be iodinated for short exposure high resolution autoradiographic studies.

Major Findings:

- (1) Estrogen and testosterone have been shown to increase oxytocin receptors in the ventromedial nucleus of the hypothalamus in males. Similarly, preliminary results with females indicate that estrogen may increase the expression of oxytocin mRNA in the rostral paraventricular nucleus of the hypothalamus.
- (2) Lesions of the paraventricular nucleus decrease maternal behavior when performed during pregnancy but not during the postpartum period. This result is consistent with earlier data implicating oxytocin in the initiation, not the maintenance, of maternal behavior.
- (3) An iodinated oxytocin analogue appears highly specific for labelling oxytocin receptors and should prove extremely useful for future studies.

Significance to Biomedical Research and the Program of the Institute: Although the past decade has seen an explosion of research in the neurobiology of cognition, locomotion, and feeding, there has been a conspicuous absence of research into the neural substrates of such primary social behaviors as mother-infant attachment, pair-bonding, and affiliative behavior. This absence seems particularly noticeable in mental health research where the inability "to love and work" has long been recognized as a common feature of diverse forms of psychopathology and early experiences of loss or isolation have been shown to affect object relations in adulthood.

Data from our laboratory increasingly point to the brain oxytocin system as an important component in the neural mediation of affiliative behaviors including parental care, sexual behavior, and social grooming. The discovery that gonadal steroids alter the expression of both the peptide and its receptors suggests that some of the behavioral effects ascribed to estrogen and testosterone may be mediated by changes in oxytocin neurotransmission. We believe that this relationship will prove to be an excellent example of a general rule--namely, that steroids in the brain regulate the genetic expression of peptides and their receptors. The elucidation of this neurobiological relationship and the description of its consequences should provide insights into a number of clinical psycho-neuro-endocrinopathies including the mental symptoms associated with postpartum and premenstrual states as well as abnormalities of parental care (e.g. child abuse and neglect).

Proposed Course: We are currently pursuing the hypothesis that oxytocin not only is involved in the onset of maternal behavior but also affects the initiation of other affiliative acts such as reproductive behavior and social grooming. Ongoing studies include measurement of brain oxytocin receptors and oxytocin mRNA across the estrous cycle to investigate the effects of physiological changes in gonadal steroids. Similar measurements in the prairie vole, an induced ovulator, will extend our work to the realm of social influences on oxytocin physiology. The connection between oxytocin and parental behavior will be investigated further by studying males that show paternal care as well as females that show maternal care that is not steroid dependent. Finally, we hope to extend our studies of parenting to birds that are brood parasites, that is, birds lacking parental care, to investigate if these species manifest different patterns of either nonapeptide mRNA or nonapeptide receptors relative to closely related species with full parental care.

Publications:

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Johnson AE, Coirini H, Ball GF, McEwen BS. Anatomical localization of the effects of estradiol-17 β on oxytocin receptor binding in the ventromedial hypothalamic nucleus. Endocrinology (in press).

Johnson AE, Coirini H, McEwen BS, Insel TR. Testosterone modulates oxytocin binding in the hypothalamus of castrated male rats. Neuroendocrinology (in press).

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NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 MH 00798-02 LCS

PERIOD COVERED

October 31, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Development of the Cerebral Cortex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. B. Stanfield Special Expert LCS NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science, NIMH

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIHAC, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

0.65

OTHER:

0.65

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This work on the development of the cerebral cortex relies heavily on neuroanatomical techniques and focuses on the role of eliminary events which occur during normal brain development. Much of our effort has concentrated on the transient visual cortical pyramidal tract which we previously identified. This normally transient projection can be at least partially maintained if the visual cortex receives somatosensory input through the lateral geniculate nucleus as a result of an induced aberrant projection of the medial lemniscus, implicating the thalamus as important in determining which projections developing cortical neurons will maintain. Collateral elimination also occurs during the development of the fornix, and of the projections of the locus coeruleus.

Our findings in experiments involving heterotopic cortical transplants implicate position within the tangential plane of the cortex as a critical factor in determining which of the initially extended projections, a cortical neuron will maintain.

Other Collaborative Professional Personnel Engaged on the Project:

D.D.M. O'Leary Assistant Professor Washington Univ. Sch. of Med., St. Louis, MO

Project Description:

Objectives: The overall goal of this project is to gain a better understanding of the development of the cerebral cortex. We have concentrated our studies on the role certain regressive or eliminatory phenomena play during normal cortical development. During the past ten years or so it has become increasingly clear that these are critical in shaping the projection patterns which are found in the adult cortex.

Methods Employed: The experiments completed or in progress can be considered in three separate groups:

1.) A characteristic feature of the organization of adult neocortical projection systems is that the cells of origin of these are arranged in discontinuous patterns within the tangential plane of the cortex. In many cases, of course, these distribution patterns reflect the underlying functional localization within the various cortical regions. It is now known that many of these discontinuous patterns emerge during development from more widespread and continuous distributions of projection neuron populations. One of the most striking examples of this is found in the development of the corticospinal projection in rats. In adult animals pyramidal tract neurons are restricted to the rostral two-thirds or so of the neocortex, in regions including the motor and somatosensory cortex. However, several years ago we reported that during the first two postnatal week pyramidal tract neurons are spread virtually throughout the tangential plane of the cortex, including the visual cortex of the occipital pole. The experiments in this first group of studies stem from that original observation and are aimed at learning more about this interesting phenomenon which seems to play a critical role in the establishment of the normal pattern of distribution of pyramidal tract neurons.

It has recently been shown that in rats, regions of primary sensory cortex (i.e. the somatosensory cortex, the auditory cortex and the visual cortex) contain a dense band of AChE activity, transiently, during the second postnatal week. Since this overlaps with the period during which the distribution of pyramidal tract neurons is widespread, we made use of this feature to positively identify these sensory regions in brains from animals in which a fluorescent retrograde marker, Fast Blue, had been injected into the pyramidal decussation, to label pyramidal tract neurons. In other rat pups, we injected an anterograde marker, WGA-HRP, into the visual cortex to study the pyramidal tract projection from the transient pyramidal tract neurons. The WGA-HRP was visualized following TMB histochemistry and additional sections were processed for AChE histochemistry to verify that the injection was restricted to visual cortex.

The observation that many cortical projection neuron populations are initially distributed widely and continuously across the cortex and the fact that, as we and others have shown, the redistribution of these involves chiefly collateral elimination rather than cell death, led us to the suggestion that the differences seen in the projections of the various regions of the adult cortex are not intrinsic to the neurons found in these regions during development. Rather it seems that cortical projection neurons initially project to several targets, but only maintain collaterals that are appropriate for their locale. Consistent with this idea is our subsequent finding that neurons within pieces of fetal occipital cortex which transplanted to the rostral cortex of a newborn host

are able to extend pyramidal tract axons and maintain these beyond the age at which occipital pyramidal tract axons are normally eliminated. We have recently shown that the pyramidal tract axons from such transplants are physiologically viable. In order to explore further the projections extended and maintained by cortical neurons transplanted to a new cortical locale, we carried out additional experiments, transplanting rostral cortex to an occipital locale as well as occipital cortex to a rostral locale, and utilizing ³H-thymidine autoradiography to identify the transplants and the retrograde tracers, Fast Blue and Diamidino Yellow, to examine the projections at different survival times and to additional targets.

Since our findings using heterotopic cortical transplants implicated extrinsic factors as having a major influence on the determination of regional cortical projections, we explored the possibility that the input the visual cortex receives through the thalamus influences which of their initially extended projections visual cortical cells will maintain. We did this by examining whether medial lemniscal axons would come to innervate the lateral geniculate nucleus if, early on, the medial lemniscus is deprived of its normal thalamic target and the lateral geniculate is deprived of its normal retinal input and then exploring whether this induced somatosensory input through the lateral geniculate would prevent the elimination of the normally transient occipital pyramidal tract axons.

Since our work has indicated the importance of the influence which the cortex receives through the thalamus on the cortical projections which eventuate, we have studied the early postnatal development of the major non-visual inputs to the thalamus using anterograde and retrograde tracers.

2.) We have used anterograde and retrograde tracing techniques to study a transient postmamillary component of the fornix which is present in rats only during development. We have used a delayed retrograde labeling paradigm to determine if the cells of origin of the postmamillary fornix survive and a delayed double retrograde labeling paradigm to determine if these cells maintain a definitive projection to the mamillary bodies.

3.) We have used retrogradely transported tracers to explore the distribution of locus coeruleus neurons which project to the hippocampus in tottering (tg/tg) mutant mice. The impetus for this study comes from our finding that during normal development the distribution of locus coeruleus cells projecting to some targets is more widespread than that found in adults and that this change results from collateral elimination. We wondered therefore if the hyperinnervation of some locus coeruleus targets in the absence of any increase in the number of locus coeruleus neurons which has been described in this mutant might be due to a maintenance of the increased numbers of coeruleus neurons innervating coeruleus targets.

Major Findings: The major findings of these three groups of studies can be summarized as follows:

1.) The use of AChE histochemistry on adjacent sections has allowed us to confirm our earlier finding that during early postnatal development in rats there are pyramidal tract neurons in the primary visual cortex which extend axons into the spinal cord. We have also shown that pyramidal tract neurons are transiently present in the developing rat auditory cortex as well.

In our experiments utilizing heterotopic cortical transplants we found that in every instance the heterotopic transplants extended and maintained projections appropriate for their new cortical locale, rather than to their site of origin. Thus, rostral cortical transplants placed in the occipital cortex only transiently extend pyramidal tract axons, just as the normal occipital

cortex does. It would seem in this instance then, that collateral elimination may essentially simply reflect a lack of regional prespecification of projections within the neocortical epithelium. Initially cells in all regions of the neocortex which are of the same projection neuron type seem to extend similar projections. The acquisition of the regional differences in cortical projections is a subsequent event achieved largely through collateral elimination and strongly influenced, if not determined, by factors extrinsic to the cortical neurons and dependent upon a cell's tangential position. Thus, in terms of the various projection neuron types the different regions initially contain, the early neocortex is a rather homogeneous structure.

We have found that at least some of the normally transient visual cortical pyramidal tract axons can be maintained if, during early postnatal development, much of the rostral cortex is lesioned. And even more visual cortical pyramidal tract axons survive if, in addition to the rostral cortical lesion, both eyes are removed. We have been able to show that such lesions also result in an abnormal innervation of the lateral geniculate nucleus by medial lemniscal fibers, and this innervation is more robust when both eyes are removed in addition to the cortical lesion than if only the cortical lesion is done. The correlation of the two effects of these manipulations and the magnitude of these effects suggests that the kind of input received through the thalamus influences which of the initially extended projections visual cortical neurons will maintain.

In our study of the development of the major non-visual thalamic afferents we found that during the early postnatal development of all these systems there is a proportion of afferent axons which extends beyond the appropriate thalamic nucleus. In some cases these errant axons even extend beyond the thalamus to enter the internal capsules. The establishment of the adult configuration of the distribution of these axonal systems proceeds over the first few postnatal weeks, and involves not only the elaboration of terminal arbors within the appropriate thalamic nucleus, but the elimination of the axons which initially extended beyond the borders of that nucleus as well. These results suggest that developmental overgrowths may be a general feature of the development of the major thalamic afferent pathways.

2.) We have found that the postmamillary component of the rat fornix is first present just before birth and before any fornix fibers entering the mamillary bodies can be identified. This postmamillary component of the fornix continues to grow into the midbrain and pontine tegmentum during the first postnatal week as the projection into the mamillary nuclei is elaborated, and then, during the second and third postnatal weeks, the postmamillary component of the fornix attenuates until it is virtually eliminated. Our experiments using retrograde tracers indicate that the cells of origin of this transient postmamillary component of the fornix are found within the subicular complex of the hippocampal region, and using a delayed retrograde labeling paradigm, we have found that most, if not all, of the cells of origin of the postmamillary component of the fornix survive the period during which this projection is eliminated. Furthermore, using a delayed double retrograde labeling paradigm we have found that many of the subicular neurons that extend transient postmamillary collaterals are cells which maintain a projection to the mamillary complex. Taken together, these observations lead us to the surprising notion that the axons which in the adult constitute the definitive projection of the fornix to the mamillary nuclei, arise during development as interstitial collaterals from trunk fibers, the distal portions of which are subsequently eliminated.

3.) We have found that in spite of the hyperinnervation of the hippocampus by the locus coeruleus in tg/tg mutant mice, the topography within the locus of the cells which give rise to this projection is normal. Thus, the hyperinnervation in this mutant apparently does not result from the maintenance of a normally transient widespread distribution of locus coeruleus projection neurons.

Significance to Biomedical Research and to the Program of the Institute: These studies have helped to establish that collateral elimination constitutes a major and widely present feature of the normal development of the central nervous system. Further, our work is beginning to provide clues to the factors which are important influences on which of the developing collaterals will be lost and which will be maintained. Our observations on heterotopic cortical transplants and on the development of the fornix have provided insights into roles collateral elimination plays as a mechanism which enable the establishing of the final configuration of neuronal connectivity, while limiting the amount of cellular prespecification necessary for this.

Proposed Course of the Project: During the following year our work will proceed along the following lines:

We will continue and complete our studies of the projections extended and those maintained by heterotopic cortical transplants made during development.

We will continue our studies of the maintenance of the occipital pyramidal tract neurons and the medial lemniscal innervation of the lateral geniculate nucleus in neonatally lesioned and enucleated rats by attempting to examine both of these events in individual animals in order to test the strength of the correlation of the two.

In order to explore what factors may be involved in its normal elimination, we will study the fate of the postmamillary component of the fornix in rats in which the mamillary bodies have undergone transneuronal degeneration following an early lesion to the cingulate cortex.

We will continue our study of the distribution of locus coeruleus projection neurons in tottering (tg/tg) mutant mice by examining coeruleospinal neurons.

Publications:

Stanfield B, Nahin BR, O'Leary DDM. A transient postmamillary component of the rat fornix during development: Implications for interspecific differences in mature axonal projections, *J Neurosci* 1987; 7: 3350-3361.

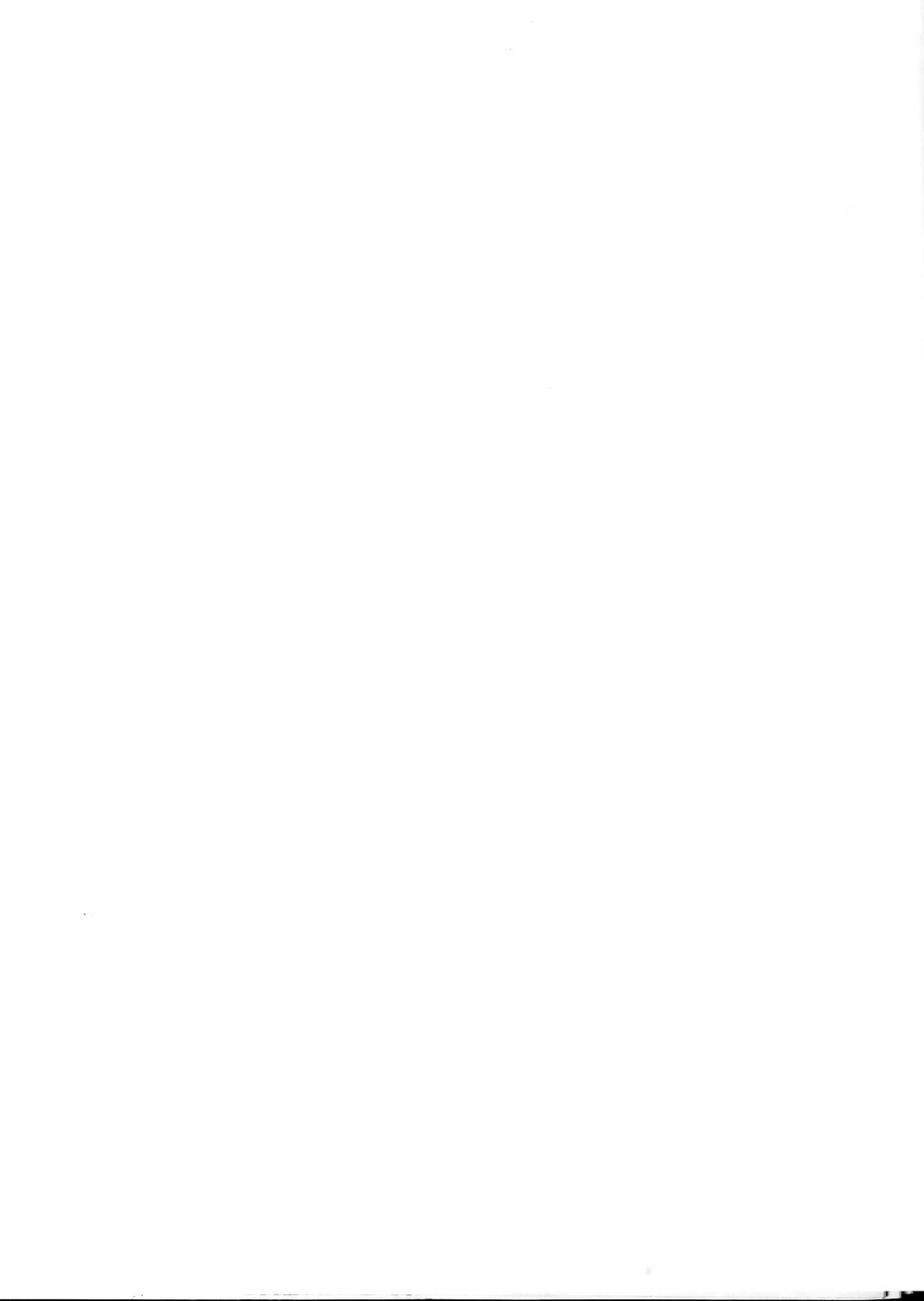
Porter LL, Cedarbaum JM, O'Leary DDM, Stanfield BB, Asanuma H. The physiological identification of pyramidal tract neurons within transplants in the rostral cortex taken from the occipital cortex during development, *Brain Res* 1987; 436: 136-142.

Stanfield BB, Cowan, WM. The development of the hippocampal region. In: Peters A, and Jones EG, eds. *Cerebral Cortex Vol. 7: The Development and Maturation of the Cerebral Cortex*. New York: Plenum Press, New York, 1988;91-131.

Asanuma C, Ohkawa R, Stanfield BB, Cowan, WM. Observations on the development of certain ascending inputs to the thalamus in rats. I. Postnatal development, *Develop Brain Res* 1988; 41: 159-170.

Stanfield BB, O'Leary DDM. Neurons in the rat subiculum with transient postmamillary collaterals during development maintain projections to the mamillary complex, *Exper Brain Res* 1988; 72: 185-190.

O'Leary DDM, Stanfield BB. Selective elimination of axons extended by developing cortical neurons is dependent on regional locale. Experiments utilizing fetal cortical transplants, *J Neurosci* (in press).



NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 MH 00799-02 LCS

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters, or less. Title must fit on one line between the borders.)

Studies of Postnatal Neurogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	B.B. Stanfield	Special Expert	LCS, NIMH
	T.R. Insel	Staff Physician	LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science, NIMH

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

0.70

PROFESSIONAL:

0.35

OTHER:

0.35

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

These Studies utilize ^3H -thymidine autoradiography, neuroanatomical tract tracing and cell counting techniques to study neuronogenesis in the adult dentate gyrus. We have found that the dentate granule cells which are generated in adult rats can extend axons through an apparently mature neuropil, and for appreciable distances. In order to determine whether new neurons are added to the adult primate brain, we are studying neuronogenesis in the dentate gyrus of pygmy marmosets.

Project Description:

Objectives: In almost all regions of the mammalian CNS, after the adult complement of neurons is generated during development, there is no residual population of neuronal precursors. Thus, when neurons are lost, due to injury or attrition, they are not replaced. However, in the rat dentate gyrus, following the perinatal surge in neuronal production, new granule cells continue to be generated at a slow rate well into adulthood. We are interested in learning more about this residual neuronal precursor pool with the eventual goal of understanding the mechanisms which control the slow accretion of dentate neurons.

Methods Employed: Recent 3H-thymidine autoradiographic evidence indicates that dentate granule cells continue to be produced at a slow yet identifiable rate throughout most, if not all, of a rat's life. To verify that the cells which incorporate the 3H-thymidine in the adult rat dentate gyrus are indeed neurons, and to determine whether these cells extend axonal projections for appreciable distances, we injected a series of animals with 3H-thymidine on postnatal day 100 and, four weeks later injected the retrograde tracer, Fast Blue, into the projection pathway of the granule cells, the mossy fiber layer of the hippocampus. After processing sections from these brains for autoradiography, we examined them under bright- and dark-field illumination, as well as fluorescence epi-illumination.

In order to determine if new granule cells are added to the dentate gyrus in an adult primate, we are using cell density measurements and volumetric determinations to estimate total cell number, as well as autoradiography to identify 3H-thymidine incorporation in pygmy marmosets (*Cebuella pygmaea*) of various ages. The pygmy marmoset was chosen due to the small brain and body size (an average adult weighs only about 150 g) and the relatively short period of time between birth and adulthood in these New World primates.

Major Findings: In autoradiograms from the rats injected with 3H-thymidine on postnatal day 100, most of the 3H-thymidine labeled cells in the dentate gyrus which were within regions containing retrogradely labeled granule cells, were, themselves, labeled with the retrograde tracer. Thus, the cells which incorporated 3H-thymidine following the injection on postnatal day 100 are indeed neurons which extend axons for considerable distances. This indicates that the new neurons which are slowly but steadily generated in the granule cell layer of the adult dentate gyrus are able to extend axons which grow and presumably establish contacts within the normal mature neuropil, adding continuously new neuronal elements to the hippocampal circuitry.

We have collected and histologically processed all of the material for our study of dentate granule cell number in pygmy marmosets of various ages and are in the process of doing the counts and measurements necessary to obtain reliable estimates of total cell numbers.

Significance to Biomedical Research and to the Program of the Institute: These studies on the continuing neuronogenesis in the adult dentate gyrus have helped to establish that, in rodents at least, new neurons are generated in the adult, that these new neuronal elements do not simply replace neurons which are lost, and that they are able to extend axonal processes and become integrated into the hippocampal circuitry. Our study of pygmy marmosets should allow us to determine if this occurs in primates as well.

Proposed Course: During the following year our work will proceed along the following lines:

We will continue and complete our study of the dentate gyrus of pygmy marmosets to determine if 3H-thymidine will be incorporated by dentate granule neurons in adults of this species and if the total number of dentate granule neurons changes significantly during the lifetime of a primate.

Publications:

Stanfield BB, Trice JE. Evidence that granule cells generated in the dentate gyrus of adult rats extend axonal projections, *Exper Brain Res* 1988; 72: 399-406.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00800-01 LCS

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

book on Brain Evolution in Relation to Paleocerebral Functions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. D. MacLean Intramural Research Scientist LCS, NIMH

Other:

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

0.7

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has involved the completion of a book entitled THE TRIUNE BRAIN IN EVOLUTION. Role in Paleocerebral Functions. In its evolution the forebrain of advanced mammals has expanded as a triune structure that anatomically and chemically reflects ancestral commonalities with reptiles, early mammals, and late mammals. Paleocerebral functions apply to those that account for species-typical behavior, including the orchestration of the daily master routine and subroutines; prosematic (nonverbal) communication; and behavior inferred to reflect factors of a compulsive and thymogenic nature. The book incorporates the author's experimental and clinical investigations so as to form part of a larger picture based on an historical perspective and relevant published work on this subject. The book begins and ends with a consideration of questions raised by brain research in regard to "epistemics" and epistemology and the human quest for a cosmic view of life.

Objectives: This year afforded completion of a book entitled THE TRIUNE BRAIN IN EVOLUTION. Role in Paleocerebral Functions. In evolution the forebrain of advanced mammals has expanded as a triune structure that anatomically and chemically reflects ancestral commonalities with reptiles, early mammals, and late mammals. Paleocerebral functions apply to those accounting for species-typical behavior, including the orchestration of the daily master routine and subroutines; prosematic (nonverbal) communication; and behavior inferred to reflect factors of a compulsive and thymogenic nature.

Methods Employed: One of the original purposes of the present laboratory was to provide a facility for investigating the functions of ganglionic structures of the basal forebrain having an anatomical and chemical correspondence in reptiles, birds, and mammals. These structures are collectively referred to as the striatal complex. Although traditionally regarded as part of the motor apparatus under control of the motor cortex, it was recognized both clinically and experimentally that there may exist large cavities in parts of the striatal complex with no apparent deficits in motor function. The possibility suggested itself that comparative neurobehavioral studies on animals living in a semi-natural environment and interacting with other animals might reveal functions of the striatal complex that otherwise would not be apparent. The resulting experimental findings indicated that the striatal complex in animals as diverse as lizards and monkeys plays an important role in the recognition and performance of displays used in social communication and also that, in conjunction with the limbic system, it provides a neural substrate for the daily master routine and subroutines.

The Poolesville facility also provided the opportunity to conduct additional studies on the functions of the limbic system. Significantly, it was found that three forms of behavior that characterized the evolutionary transition from reptiles to mammals--namely, nursing in conjunction with maternal care; audiovocal communication for maintaining maternal-offspring contact; and play--are represented in the thalamocingulate division of the limbic system. This evolutionarily newest part of the limbic system has no distinctive counterpart in the reptilian brain.

Major Findings: In the just completed book, the foregoing studies, together with other past experimental and clinical investigations involving the principal investigator, have been incorporated into a broader canvas giving a perspective of the historical, anatomical, functional, behavioral, and psychological aspects of the subject. The book begins and ends with a consideration of questions raised by brain research in regard to "epistemics" and epistemology and the human quest for a cosmic view of life. The chapters of the book are listed on the following page.

Significance to Biomedical Research and the Program of the Institute: The book is relevant to mental health insofar as it attempts to provide new insights into the evolution of paleopsychic functions of the brain that may prove helpful in the understanding and interpretation of inarticulate mental conditions. It also calls attention to the need, scientifically, for a greater knowledge of the workings of the subjective brain, an aspect of epistemology referred to as "epistemics."

Proposed Course: To be completed upon publication of book.

THE TRIUNE BRAIN IN EVOLUTION

Role in Paleocerebral Functions

Introduction

1. Towards a Knowledge of the Subjective Brain ("Epistemics")
2. Specific Indications for Brain Research
3. Role of Forebrain Contrasted with that of the Neural Chassis

Part I. The Striatal Complex with Respect to Species-typical Behavior

4. Evolutionary Considerations, Anatomy, and Question of Function
5. The Mammal-like Reptiles (Therapsids)
6. Reptilian Behavior as Typified by Lizards
7. A Day in the Life of a Rainbow Lizard
8. A Week in the Life of a Giant Komodo Dragon
9. Other Special Forms of Basic Behavior
10. Six General Forms of Basic Behavior
11. Neurobehavioral Findings on a Lacertian Display
12. Avian Display
13. Role of R-complex in Display of Squirrel Monkeys
14. Further Evidence Implicating Striatal Complex in Display Behavior
15. Some Relevant Clinical Findings
16. Human-related Questions

Part II. The Limbic System with Respect to Thymogenic Functions

17. The Limbic System in Historical Perspective
18. An Anatomical Framework for Considering Limbic Functions
19. Functions of Amygdalar and Septal Divisions with Respect to Self-preservation and Procreation
20. Participation of Thalamocingulate Division in Limbic Sex-related Functions
21. Functions of the Thalamocingulate Division in Family Related Behavior
22. Phenomenology of Psychomotor Epilepsy with Respect to Classification and Cerebral Representation of Affects.
23. Phenomenology of Psychomotor Epilepsy in Regard to Basic and Specific Affects
24. Phenomenology of Psychomotor Epilepsy in Regard to General Affects
25. Automatisms of Psychomotor Epilepsy with Respect to Prototypical and Emotional Behavior
26. Microelectrode Study of Limbic Inputs Relevant to Ontology and Memory
27. Question of Limbic Mechanisms Linking a Sense of Individuality to Memory of Ongoing Experience

Part III. Neo-encephalon in Connection with Paleocerebral Functions

28. Neocortex, with Special Reference to the Frontal Granular Cortex

Conclusion

29. Implications for Future Thinking in Regard to Epistemics and Epistemology

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00851-24 LCS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Mechanisms of Display Behavior in Squirrel Monkey (*Saimiri sciureus*)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.D. MacLean, M.D. Section on Comparative Studies of Brain and Behavior LCS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Work on this project to be completed with the publication of a final monograph.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 MH 02219-05 LCS

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Animal Models of Anxiety

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T.R. Insel
J.L. WinslowStaff Physician
IRTA FellowLCS, NIMH
LCE, NICHD

COOPERATING UNITS (if any)

Laboratory of Comparative Ethology, NICHD; Addiction Research Center, NIDA, Baltimore, MD

LAB/BRANCH

Laboratory of Clinical Science, NIMH

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.7

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the fifth year of this project, our focus continues to be the ontogeny of stress response systems in the brain with a special reference to the role of these systems in the organization of behavior. Last year we described the early and exuberant development of brain corticotropin releasing factor (CRF) receptors in the rat. During this year, we investigated the role of these receptors in the rat pup's behavioral response to social isolation. Central (i.e. intracerebroventricular), but not peripheral, administration of CRF was found to decrease the number of ultrasonic isolation calls in the 6-day-old rat. The number of these calls was increased following central administration of a synthetic CRF antagonist. In related studies, central administration of the same peptides to squirrel monkeys revealed dose-dependent changes in locomotor behavior as well as in several species-typical patterns of vigilance and aggression.

We also studied the effects of stress at different stages of development on CRF receptors. Opiate receptors were also measured as these same stress paradigms have been associated with hypoalgesia. No alterations in brain CRF receptors were noted although μ opiate receptors decreased in selective regions following stress in either the prenatal or adult periods.

Taken together, these results implicate brain CRF receptors in the mediation of several stress-related behaviors. In spite of our earlier discovery of profound changes in CRF receptor number and distribution in development, these new results suggest that the numbers of these receptors, either in adulthood or infancy, are not altered by several forms of environmental stress.

Other Collaborative Professional Personnel Engaged in the Project:

Craig Kinsley	Asst. Prof.	Lab. Reprod. Biol.	Harvard Med. School
Thomas Minor	Asst. Prof.	Brain Res. Inst.	UCLA

Project Description:

Objectives: During this year, we extended our previous investigations into the ontogeny of brain corticotropin releasing factor (CRF) by studying the behavioral responses to centrally administered CRF in rat pups. The major question here was: would CRF have stress related effects in development? In a related study, last year, we were unable to identify long-term or organizational consequences of repeated CRF administration to rat pups probably due to the failure of peripherally administered CRF to reach brain receptors. As stress might release endogenous CRF with resulting alterations in the number or distribution of receptors, we investigated several stress paradigms in rats at various stages of development to determine if stress would alter brain CRF or opiate receptors. In addition, we began an extensive study of the behavioral effects of CRF administered centrally to squirrel monkeys, with the eventual goal of comparing behavioral responses in subordinate and dominant members of a social group.

Methods Employed: The infant response to brief separations has been investigated in rat pups from 1 to 14 days of age. Rat pups, when separated at these ages, emit ultrasonic calls which can be detected, quantified, and characterized using a computer-based sound spectrum analyzer. Our studies have investigated the pharmacologic modification of these calls by testing pups isolated for 2 minutes prior to peptide administration, replacing the pup in its litter for 30 minutes, and retesting during a second 2-minute isolation period. In nonpharmacologic studies we have previously investigated the influence of several other factors such as temperature, age, and presence of littermates on production of ultrasonic isolation calls.

The effects of various peptides on ultrasonic calls have been investigated by direct intracerebroventricular injections using 10% ink to mark the injection site at necropsy. This method is not traumatic even in unanesthetized pups in the first week of postnatal life before the skull is calcified. With this technique both CRF and the synthetic antagonist, α -helical CRF, were studied.

Stress paradigms at various stages of development were used. Prenatal stress employed daily brief restraint of pregnant dams from days 15-21 of gestation. Postnatal stress was either 2 or 6 hours of social isolation daily for days 1-10. Adults were studied following a standard inescapable shock or "learned helplessness" paradigm. Developmentally stressed animals were sacrificed at day 42 and prepared for either μ opiate receptor (^3H -DAGO) or CRF receptor assay. Learned helpless animals, used in similar binding studies, were at least 120 days old.

We administered either CRF or the CRF antagonist, α -helical CRF₍₉₋₄₁₎ to squirrel monkeys via chronic indwelling intracerebroventricular cannulae. Animals were studied under three conditions: social isolation, mirror presentation, and alarm test (presentation of

a puppet). Behavior was videotaped for subsequent scoring. In addition, animals wore portable activity monitors to determine CRF effects on behavioral activity.

Major Findings:

(1) The stress related peptide CRF decreases the behavioral response to social isolation in rat pups when administered centrally but not peripherally. The CRF antagonist, α -helical CRF blocks this effect and, when given by itself, increases the number of isolation calls. These paradoxical responses suggest that stress-response systems in the brain function very differently during infancy.

(2) CRF receptors in rat brain are not altered by isolation stress in development or the learned helplessness paradigm during adulthood. In contrast to CRF, μ opiate receptors appear sensitive to both interventions. Following the learned helplessness paradigm and concurrent with the analgesia previously reported, we found a significant decrease in opiate receptors in the midbrain, although not in four other brain regions. Rats stressed *in utero* have a significant decrease in μ receptors in striatum and midbrain but not in cortex, thalamus, or hypothalamus.

(3) CRF administered centrally to squirrel monkeys is associated with dose dependent increases in measures of activity and vigilance. The CRF antagonist blocked these effects, and in the mirror test condition (conspecific threat), appeared to have intrinsic effects increasing aggressive threat.

Significance to Biomedical Research and the Program of the Institute: Two findings are of particular relevance to clinical research. First, these results argue against a simple characterization of CRF as a "stress peptide." The behavioral effects of CRF depend on the stage of development, the social context, and possibly the social status.

A second finding of importance was the demonstration of long-term alterations in opiate receptors following stress during development. These receptor changes are associated with behavioral effects and thus provide a useful model for the major principle that has guided this project for the past two years: that within development there are sensitive periods during which the long-term regulation of neuro-receptors is determined. We believe this principle is relevant to the clinical observation that loss during human infancy predisposes to dysregulated affective and neuroendocrine systems in adulthood.

Proposed Course: In the coming year we plan to wind down our studies of CRF receptors while expanding our behavioral studies of CRF effects in socially housed squirrel monkeys. In addition, we will further investigate the mechanisms of long-term alterations of opiate receptors following prenatal stress. Finally, studies currently underway will extend the same strategy to excitatory amino acid and benzodiazepine receptors--mapping the ontogeny of receptors and exploring environmental influences on ultimate receptor number and distribution.

Publications:

De Souza EB, Insel TR. Brain distribution of CRF receptors. In: De Souza E, Nemeroff C, eds. Corticotropin Releasing Factor: Basic and Clinical Aspects. New York: CRC Press, in press.

Glowa JR, Bergman J, Insel T, Newman JD. Drug effects on primate alarm vocalizations. In: Newman JD, ed. The Physiological Control of Mammalian Vocalization. New York: Plenum Press, 1988;343-366.

Insel TR. Decreased *in vivo* binding to the brain benzodiazepine receptor during social isolation. Psychopharmacology (in press).

Insel TR. Corticotropin releasing factor in development. In: De Souza EB, Nemeroff C, eds. Corticotropin Releasing Factor: Basic and Clinical Aspects. New York: CRC Press, in press.

Insel TR, Battaglia G, Fairbanks DW, De Souza EB. The ontogeny of brain receptors for CRF and the development of their functional association with adenylate cyclase, J Neurosci (in press).

Insel TR, Gelhard RE, Miller LP. Rat pup isolation distress and the brain benzodiazepine receptor, Develop Psychobiol (in press).

Insel TR, Miller LP, Gelhard RE, Hill JL. The neural basis of the rat pup ultrasonic isolation calls. In: Newman JD, ed. The Physiological Control of Mammalian Vocalization. New York: Plenum Press, 1988;331-342.

Insel TR, Scanlan J, Champoux M, Suomi SJ. Rearing paradigm in a nonhuman primate affects response to β -CCE challenge. Psychopharmacology 1988;96:81-86.

Insel TR, Wamboldt MZ. Pharmacologic models of anxiety. In: Handbook of Anxiety. New York: Pergamon Press, 1988;181-216.

Margangos PJ, Insel TR, Montgomery P, Tamborska E. Brain adenosine receptors in Maudsley reactive and non-reactive rats, Brain Res 1987;421:69-74.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00382-14 LCS
PERIOD COVERED <p style="text-align: center;">October 1, 1987 to September 30, 1988</p>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <p style="text-align: center;">Localization and Characterization of Brain Neurochemicals</p>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
David M. Jacobowitz	Chief, Histopharmacology Section	LCS, NIMH
Bryan L. Roth	Capt., Surgical Research Branch	SRB, NMRI
Michael J. Iadarola	Visiting Associate	LNA, NIDA
COOPERATING UNITS (if any) <p style="text-align: center;">Surgical Research Branch, Naval Medical Research Institute</p>		
LAB/BRANCH <p style="text-align: center;">Laboratory of Clinical Science</p>		
SECTION <p style="text-align: center;">Histopharmacology</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NIMH, ADAMHA, Bethesda, Maryland 20892</p>		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.3	.4
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) <p>This study describes a simple technique for purifying protein kinase C (PKC) to homogeneity and producing antibodies to specific domains of PKC subtypes using synthetic antigens. We also detail a method which allows for quantification of PKC within discrete brain regions.</p> <p>Antibodies directed against the β-PKC disclosed a heterogenous distribution in rat hippocampus with intense labeling of fibers in the s. oriens adjacent to the pyramidal cells as well as in the s. radiatum. Analysis of micropunches of rat hippocampus (300 μm each) by Western blots and visualization of PKC by antibodies labelled with [125I]-protein A showed the β-PKC to be preferentially located in the CA1 region.</p> <p>The β-PKC antibody was used to determine the distribution of β-PKC in rat hippocampus by immunocytochemistry. PKC was distributed primarily in the stratum oriens and radiatum of the CA1. Fibers were observed enveloping the pyramidal cells which were unstained. The discrete localization of various subtypes of PKC should provide clues to their functions.</p>		

Project Description

Objectives: (1) Preparation of antibodies against a β -type PKC synthetic peptide sequence. (2) Develop a procedure for quantifying the relative amounts of PKC in discrete brain regions. (3) Immunocytochemical mapping of the β -subunit of PKC within the hippocampus.

Methods Employed: 1) Rabbit antibody production; 2) Gel electrophoresis; 3) Micropunch of discrete brain regions; 4) Western blotting; 5) Immunocytochemistry.

Major Findings:

1) Rabbit antibodies prepared against a β -type PKC synthetic peptide sequence showed high specificity and sensitivity for PKC and recognized only the 78 kD form of PKC.

2) Micropunches of rat hippocampal subregions were solubilized in SDS sample buffer, run on 10% SDS polyacrylamide gels and transferred to nitrocellulose. PKC was visualized by [125 I]protein A autoradiography and quantified by densitometry. The highest concentrations of PKC were found in the CA1-pyramidal cell layer (0.43 ± 0.04 OD) with the lowest amounts in the CA3 and CA4 pyramidal cell layers (0.11 ± 0.02 and 0.085 ± 0.006 OD respectively).

3) Immunohistochemical studies revealed that PKC was distributed primarily in the stratum oriens and radiatum of the CA1 region. Fibers were observed enveloping the pyramidal cells which were unstained.

Significance to Biomedical Research and the Program of the Institute: Protein kinase C is a calcium and phospholipid-dependent enzyme which is highly enriched in rat hippocampus. Many hormones and neurotransmitters may activate PKC via production of diacylglycerol from phosphoinositide hydrolysis. Activation of PKC induces a wide variety of biochemical actions including ion-channel opening and closing, long-term potentiation and alterations of PI metabolism. The mapping of the discrete brain localization of PKC will help to better understand the functions of the multiple forms of PKC.

Proposed Course of the Project: Other subtypes of PKC will be studied in addition to further mapping of the distribution of the β -subunit in the brain.

Publications:

Jacobowitz, DM, Hara, H and Kobayashi, S: Autonomic innervation of the major blood vessels of the brain. In: Edvinsson L, McCulloch J, eds. Peptidergic mechanisms in the cerebral circulation. Ellis Horwood Ltds., 1987;48-63.

Kostrzewa, RM, Hardin, JC and Jacobowitz, DM: Destruction of cells in the midportion of the locus coeruleus by a dorsal bundle lesion in neonatal rats. Brain Res 1988;442:321-328.

Sutin, EL and Jacobowitz, DM: Immunocytochemical localization of peptides and other neurochemicals in the rat laterodorsal tegmental nucleus and adjacent area. J Comp Neurol 1988;270:243-270.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00388-12 LCS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Coexistence of Peptides and Neurotransmitters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David M. Jacobowitz Chief, Histopharmacology Section LCS, NIMH

Gerhard Skofitsch Guest Worker LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.6

PROFESSIONAL:

1.2

OTHER:

.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Immunohistochemical studies have revealed that galanin (GAL) and vasopressin (VP) coexist in the very same cell bodies in the supraoptic and magnocellular paraventricular nuclei and the accessory cells of the lateral hypothalamic area. Dehydration and salt loading, which is known to cause release and depletion of VP and oxytocin from the neurohypophysis, also caused a marked reduction of GAL-like immunoreactivity in the posterior lobe of the pituitary. Effects of systemically administered GAL caused a short lasting small increase in blood pressure with no effect on heart rate. A thousand-fold molar concentration of GAL, compared to VP, was required to cause comparable effects on blood pressure. GAL had no modulatory effect on VP-induced response. Systemically administered GAL resulted in a mild increase in diuresis whereas VP caused complete and sustained inhibition of diuresis. GAL had no effect on VP-induced antidiuretic effects. The significance of the coexistence of GAL and VP remains to be elucidated.

Project Description:

Objectives: 1) To investigate a possible coexistence of galanin (GAL) and vasopressin (VP). 2) To investigate a possible influence of GAL on diuresis in the periphery.

Methods Employed: 1) Immunocytochemistry; 2) Dehydration and salt loading; 3) Measurement of diuresis.

Major Findings:

(1) GAL-like immunoreactivity was found to coexist with VP-immunoreactivity in cell bodies of the supraoptic and magnocellular subdivisions of the paraventricular nucleus and the magnocellular accessory cell clusters.

(2) GAL- and VP-immunoreactive nerves were contained in the neural lobe of the pituitary. Following dehydration and salt loading a dramatic decrease of both GAL- and VP-immunoreactivity in the posterior pituitary was observed, whereas the hypothalamic cell body groups and neurons appeared visibly unaltered if compared to controls.

(3) Intravenous injections of GAL and VP caused an increase in blood pressure. However, VP had more than a thousand-fold molar activity than GAL. Both peptides had little or no effect on heart rate. VP induced pressor responses remained unaltered by constant infusion of GAL.

(4) GAL in high doses was slightly diuretic after s.c. or i.v. administration whereas VP markedly decreased diuresis following subcutaneous administration or caused complete and sustained inhibition of diuresis after i.v. administration. No effect of a constant infusion of GAL on i.v. administered bolus injection of VP was found.

Significance to Biomedical Research and the Program of the Institute: The coexistence of GAL and VP in the supraoptic and paraventricular nuclei is yet another example of the ever increasing reports of neuronal sites containing classical neurotransmitters coexisting with peptides. The widespread distribution of GAL in the central and peripheral nervous system suggests multiple physiological activities of this novel peptide.

Proposed Course: In view of the multiple coexistence of various peptides in the magnocellular portion of the paraventricular nucleus, it would be of interest to ascertain whether GAL is involved in the modulation of peptides and/or other neurotransmitters contained in identical or adjacent fibers in the median eminence. Such a mechanism would imply that GAL released at this level might influence anterior pituitary hormonal release.

Publications:

Skofitsch G, Jacobowitz DM and Lembeck F: Galanin and vasopressin coexist in the rat hypothalamo-neurohypophyseal system. Neuroendocrinology; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00396-10 LCS
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) A Study of Proteins Within the CNS by Two-Dimensional Gel Electrophoresis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
David M. Jacobowitz	Chief, Histopharmacology Section	LCS, NIMH
Anne-Marie O'Carroll	Visiting Fellow	LCS, NIMH
Lois Winsky	IRTA Fellow	CNB, NINCDS
Jitendra Patel	Visiting Associate	BPB, NIMH
COOPERATING UNITS (if any) Clinical Neurosciences Branch, DIRP, NINCDS		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Histopharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205		
TOTAL MAN-YEARS: 3.4	PROFESSIONAL: 2.4	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The identification of interesting proteins within the CNS utilizing 2-dimensional gel electrophoresis (2DE) continues. 1) We have isolated and purified a soluble protein designated "protein 38" which is found in the rat brain as one of the protein spots appearing on the 2DE gels. Protein 38 has a molecular weight of 57 kD (dimer) and 28.6 kD (monomer). Immunocytochemical studies using an antibody raised to this protein revealed that protein 38 is localized in capillary endothelial cells in the brain and periphery. Cell cultures derived from newborn rat cortices revealed positive staining of stress fibers within endothelial cells. 2) A second study involves the isolation, partial purification and immunohistochemical localization of a 94 kD phosphoprotein ("protein 94"). This cytosolic protein has been partially purified from the bovine caudate nucleus. Purification was monitored by autoradiography of ³²P-phosphorylated samples separated by SDS-PAGE. A rabbit antibody to this protein was used for a study of the immunohistochemical localization of this protein in cell cultures of astroglia of newborn rat cortices and revealed a single type of cell which has been tentatively identified as microglia. 3) Studies of proteins within auditory nuclei indicated one protein (MW = 29 kD, pI = 5.3) which is highly localized in the cochlear nuclei in rabbits, rats and guinea pigs. </p>		

Project Description:

Objectives: 1) To isolate and purify a protein ("protein 38") that exists on our brain gels. 2) To isolate and purify a 94 kD phosphoprotein and raise antibodies to the protein. 3) To identify proteins specifically localized to auditory regions of brain. To further characterize the cochlear nucleus protein. For example, to determine whether the cochlear specific protein is associated with inputs from the auditory nerve or is a component of other intrinsic or extrinsic cochlear cells.

Methods Employed: 1) Biochemical separation techniques - ammonium sulfate precipitation; ion-exchange and gel filtration chromatography; 2) Immunization of rabbits for the production of antiserum; 3) Immunocytochemical methods; 4) Radiolabel phosphate (from ATP) incorporation into proteins from microdissected brain tissue; 5) One and two-dimensional gel electrophoresis; 6) Silver staining of proteins on polyacrylamide gels; 7) Electrophoretic transfer of proteins to nitrocellulose paper and subsequent identification of proteins by use of specific antisera; 8) Peptide mapping using *S. aureus* V8 protease and trypsin; 9) Phosphoamino acid analysis using high voltage electrophoresis; 10) Cell culture of astrocytes and endothelial cells.

Major Findings:

(A) We have isolated and purified a soluble protein designated "protein 38" which is found in the rat brain as one of the protein spots appearing on the 2DE gels. Protein 38 is a homodimer with a molecular weight of 57,000 Daltons and a monomeric weight of 28,600 indicating that the protein might be composed of two identical subunits. Immunocytochemical studies using a rabbit antibody raised to this protein revealed that protein 38 is localized in capillaries in all regions of the brain. Capillary immunofluorescence was also revealed in vibratome sections of the kidney and liver. Cell cultures of newborn rat cortices were studied. The antiserum revealed positive staining of stress fibers within endothelial cells.

(B) The phosphoprotein has been purified to approximately 70-80% homogeneity from bovine caudate-putamen. Initial characterization studies of the protein have been carried out. Subcellular fractionation studies have shown the 94 kD protein to be localized predominantly to the cytosolic fraction. Using two-dimensional gel electrophoresis, the pI value of the phosphorylated denatured form of the protein was found to be approximately 4.7 while that of the unphosphorylated form of the protein was found to be approximately 3.8 by isoelectric focusing column chromatography. *S. aureus* V8 protease digestion of the 94 kD protein results in the progressive disappearance of the protein and the production of six well resolved cleavage fragments. This pattern of peptide is characteristic of the protein substrate and the proteolytic enzyme used. Phosphoamino acid analysis of the protein showed it to be phosphorylated solely upon serine residues.

Cell cultures of astroglia of newborn rat cortices were studied. The antiserum to the 94 kD protein immunostained a single type of cell which has been tentatively identified as microglia.

(C) A protein previously found to be greatest in amounts in the lateral superior olive (LSO) as compared with auditory nuclei was identified as the vitamin D-dependent calcium binding protein. Another protein having greatest

amounts in the cochlear nuclei and the LSO was identified as the glial fibrillary acidic protein. A protein previously found to be highly localized to the cochlear nuclei in rabbits was similarly localized to the cochlear nuclei in rats and guinea pigs.

Significance to Biomedical Research and the Program of the Institute: A major purpose of our investigation of proteins has been to establish immunochemically defined markers for neural cells and other cell structures. Underlying this approach has been the basic premise that antibodies can be valuable tools for studying a wide variety of biological systems including the nervous system. Measurement in blood and cerebrospinal fluid may provide new tools for diagnosis and monitoring of neurological diseases in addition to further immunohistochemical studies of pathological nervous tissue.

Specific antigens and antibodies to endothelial and neuroglial cells may serve as potential markers for pathological changes in the brain and CSF and therefore has potential clinical and diagnostic significance. The development of antibodies to proteins is significant in advancing our knowledge of cellular morphology and function in the brain and periphery.

The identification, purification and mapping of proteins specifically localized to components of the auditory system may be of importance for understanding auditory function and could provide clues towards identifying biochemical correlates of congenital and toxicity related central hearing loss.

Proposed Course of the Project: Isolation and purification of unknown proteins will continue. Attempts will be made to identify protein sequences of the capillary protein (protein 38), the microglia protein (protein 94) and the corticospinal protein (protein 36, described last year, Fukuda and Jacobowitz, 1988). Studies examining phosphoproteins within the auditory nuclei will continue.

Publications:

Fukuda T and Jacobowitz DM: Purification and immunocytochemical detection of a protein that reveals layer V pyramidal cells in the rat cortex.. *Brain Res* 1988;441:185-194.

Heydorn, W.E., Creed, G.J., Patel, J. and Jacobowitz, D.M.: Distribution of phosphoproteins in microdissected areas of the rat brain studied by two-dimensional gel electrophoresis. *J. Neurochem.*, in press.

Heydorn WE and Jacobowitz DM: The use of two-dimensional gel electrophoresis to study proteins in the central nervous system. In: Marangos PJ, Cohen RM eds. *Neuronal and glial proteins*. New York: Academic Press, in press.

Rodriguez-Sierra JF, Heydorn WE, Creed GJ and Jacobowitz DM: Incorporation of amino acids into proteins of the hypothalamus of prepubertal female rats after estradiol treatment. *Neuroendocrinology* 1987;45:459-464.

Santer DM, Heydorn WE, Creed GJ and Jacobowitz DM: Localization of Ca^{+2} binding proteins in rat cortex on two-dimensional gels. I. Identification of calmodulin and the B subunit of calcineurin. *Neurochem International* 1988;12:215-223.

Santer DM, Heydorn WE, Creed GJ, Fukuda T and Jacobowitz DM: Localization of Ca^{+2} binding proteins of rat cortex on two-dimensional gels. II. Analysis of Ca^{+2} binding proteins in ammonium sulfate fractions of rat brain. Neurochem International 1988;12:225-236.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00397-10 LCS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Autoimmune Aspects of Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

James S. Frazier	Staff Fellow	LCS, NIMH
Hiroyasu Nakata	Visiting Scientist	LCS, NIMH
David M. Jacobowitz	Chief, Histopharmacology Section	LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

1.1

OTHER:

.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The study of autoimmunity and disease continues with an emphasis on type 1 diabetes mellitus. The antigen which was recognized by an autoantibody, found in a small percentage of patients with type 1 diabetes, is chymotrypsin, or a "chymotrypsin-like" protein, as determined by amino acid partial sequence analysis and amino acid composition analysis. Affinity column preabsorption of antibodies raised against chymotrypsin delineated two antibody populations. With immunofluorescence microscopy, one population of antibody reacted with the monkey exocrine pancreas while the other reacted with the islets of langerhans. Western immunoblot analysis of the two populations revealed that the antibodies reacting with the exocrine pancreas recognized chymotrypsin or the "chymotrypsin-like" protein, while the population reacting with the islets reacted with a tri-isomeric, soluble protein with pI's ranging from 6.8-7.0. This protein was purified, but amino acid sequence analysis of it has so far been blocked. Antibody formation against this protein and new attempts, after partial digestion, to sequence this protein are in progress. The possibility that an idiotypic/anti-idiotypic relationship between the two populations of antibodies has been raised. Functional assays to test this possibility are in progress.

Project Description

Objectives: 1) To continue studying the autoimmune aspects of clinical disease with an emphasis on type 1 diabetes mellitus. 2) To identify antigens targeted by autoantibodies through the use of amino acid partial sequencing and amino acid composition analysis techniques. 3) To study the interactions of antibodies with other antibodies, i.e., idiotypic/antidiotypic interactions. 4) To study the pathologic significance of antibody/antigen interactions. 5) To refine autoantibody analysis techniques such that the autoimmune aspects of disease involving the CNS, including Alzheimer disease and CNS lupus, may be facilitated.

Methods Employed:

1) Two-dimensional gel electrophoresis. 2) Silver staining of proteins on gels. 3) Electrophoretic transfer of proteins to nitrocellulose paper and subsequent immunoblotting with sera or CSF. 4) Ultracentrifugation, ammonium sulfate fractionation, cation exchange chromatography, hydroxylapatite chromatography. 5) Antibody formation via rabbit immunization. 6) Antibody purification through affinity column preabsorption. 7) Immunofluorescence microscopy. 8) Amino acid composition analysis. 9) Amino acid partial sequencing. 10) Enzyme assay studies.

Major Findings:

1. The amino acid composition of the previously reported 25 kD pancreatic protein, which is recognized by antibodies in the sera of a small number of patients with Type 1 diabetes mellitus, is very similar to the composition of bovine chymotrypsin.

2. A ten residue amino acid sequence of this protein has 90% homology with a ten residue segment of bovine chymotrypsinogen.

3. Improved fixing techniques for tissue fluorescence microscopy demonstrated that the antibodies produced by the injection of the 25 kD protein in the rabbit had reactivities against both endocrine and exocrine pancreas of the monkey.

4. Affinity column preabsorption of these antibodies on a column with chymotrypsin fixed to the gel matrix revealed there to be two major groups of antibodies in the rabbit serum.

5. The antibodies which bound to the chymotrypsin affinity column, when eluted, reacted with the exocrine pancreas as revealed by fluorescence microscopy. The antibodies which did not bind to chymotrypsin and flowed through the column reacted with the islets of langerhans.

6. Western immunoblot analysis of these two groups of antibodies demonstrated that the exocrine staining antibodies recognized the 25 kD pancreatic protein while the islet staining antibodies recognized a trisomeric 50 kD pancreatic protein with pI's of pH 6.8-7.0.

7. This 50 kD protein is soluble and precipitates at an ammonium sulfate concentration of 40%.

8. After purification, this protein is blocked and therefore resistant to partial amino acid sequencing.

9. Enzymatic assays utilizing bovine chymotrypsin as the enzyme were not inhibited by the antibodies raised against the 25 kD human "chymotrypsin-like" protein.

Significance to Biomedical Research and the Program of the Institute: The finding that a small percentage of patients with type 1 diabetes mellitus have in their serum autoantibodies which react with chymotrypsin or a "chymotrypsin-like protein" may shed light on the pathogenesis of this disease. More specifically, this observation may be related to the finding that when type 1 diabetes is chemically induced in the rat with the drug streptozotocin, insulin secretion drops while chymotrypsin secretion increases. Thus, an autoantibody against chymotrypsin may alter normal endocrine-exocrine interactions. Alternatively, an autoantibody directed against an isomeric form of chymotrypsin in the islets themselves may be directly involved in the destruction of these tissues.

Sequencing the 50 kD islet-related protein would be of general interest. If cloning and sequencing reveals that the 50 kD protein is a known protein with specific roles in insulin production or islet cell function, direct implications regarding the autoimmune component of type 1 diabetes could be made.

Finding populations of antibodies, from the same bleed, which react separately with the endocrine and exocrine pancreas may point towards interesting relationships between the two tissues. It is conceivable that one of these antibodies was formed first and then later caused the formation of the other antibody, i.e., an "anti-idiotypic" antibody was formed in response to the original "idiotypic" antibody. Continued use of functional assays, but with species matched enzymes and substrates, will help prove the viability of this possibility.

In general, the study of idiotypic/anti-idiotypic interactions is potentially very important in the study of the immune components of CNS disease. Anti-receptor autoantibodies have been documented in peripheral nervous system and endocrine disease. Similar work related to CNS disease is beginning to emerge. Continued study of the specifics of idiotypic and anti-idiotypic antibody formation and the possible interrelationships of these antibodies and target antigens (receptors and neurotransmitters) may be very helpful in resolving some of the complexities of CNS disease.

Proposed Course of the Project: 1) Sequence and clone 50 kD islet-related protein. 2) Continue studying possible idiotypic/anti-idiotypic interactions. 3) Continue examining the autoimmune aspects of CNS disease by looking for possible anti-idiotypic and anti-receptor antibodies in disease such as Alzheimer's and CNS Lupus.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02377-02 LCS
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) A Study of Adenosine Receptors: Isolation and Characterization		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Hiroyasu Nakata	Visiting Scientist	LCS, NIMH
David M. Jacobowitz	Chief, Histopharmacology Section	LCS, NIMH
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Histopharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.3	PROFESSIONAL: 1.1	OTHER: .2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>Isolation of A1 and A2 adenosine receptors from rat brain membranes has been continued. It was found that these receptors could be separated from each other by hydroxylapatite chromatography. It was also found that another adenosine binding protein which showed similar but distinguished properties from A2 receptors was present in the digitonin-solubilized rat brain membranes and could be separated from adenosine receptors by hydroxylchromatography. Moreover, a new affinity chromatography system for the purification of A1 receptor was developed and A1 receptor of the crude solubilized membrane preparation was purified to ~ 50-fold by this method.</p>		

Project Description:

Objectives: 1) To isolate and characterize the adenosine receptors from rat brain.

Methods Employed:

1) Ultracentrifugations for subfractionation of rat brain membranes and for isolation of solubilized receptor fraction from the digitonin-treated membranes. 2) Column chromatographies of hydroxylapatite, affinity gels or other type of gels. 3) HPLC and FPLC. 4) Radiolabeled ligand binding assay using cell harvester. (5) Chemical coupling of adenosine antagonists to support gels.

Major Findings:

(A) A new affinity chromatography system was developed. The affinity gel was synthesized by coupling xanthine amine congener, a potent adenosine antagonist, with Affi-Gel 10 using hydroxysuccinimide ester reaction. The solubilized A_1 receptor from rat brain membranes was applied to the affinity gel column and specifically eluted with 8-cyclopentyltheophylline. The adsorption and elution of A_1 receptor were shown to be biospecific. The affinity purification resulted in an approximately 50-fold purification with a recovery ~ 30% of the solubilized A_1 receptor applied on the column. This affinity chromatography was also applied successfully for the purification of rat testicular A_1 receptor.

(B) The solubilized adenosine receptors of rat brain membranes were separated into two major peaks, i.e., A_1 and A_2 receptors, by hydroxylapatite chromatography in the presence of 100 mM NaCl.² It was also found that an unknown non-receptor adenosine binding protein could be separated from the adenosine receptor protein by hydroxylapatite chromatography in the absence of NaCl.

Significance to Biomedical Research and the Program of the Institute: Adenosine and its stable analogs are known to show various physiological effects on many tissues including the nervous systems. These include modulation of adenylate cyclase, inhibition of both nerve cell firing and neurotransmitter release in vivo and in vitro and a sedative action. Most of these actions are mediated via adenosine receptors. Therefore, it is important to characterize the biochemical properties of the adenosine receptor in order to understand the function of adenosine which has a variety of pharmacological effects.

Proposed Course: Isolation and purification studies will continue.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00153-11
CHP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Treatment of Obsessional Children and Adolescents with Clomipramine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith L. Rapoport, M.D., Chief, CHP, NIMH

Henrietta Leonard, M.D., NRSA Fellow, CHP, NIMH

Dennis P. Murphy, M.D., Chief, LCS, NIMH

Theodore Zahn, Ph.D., Research Psychologist, LPP, NIMH

Agnes Whitaker, M.D., Columbia University

Susan Swedo, M.D., Staff Fellow, CHP, NIMH

Martha Denckla, M.D., Chief, Autism & Behav. Dis. Sect., DNB, NINCDS

Mark Shapiro, M.D., LN, NIA

COOPERATING UNITS (if any)

Lab. of Psychol. & Psychopathology, NIMH; Clin. Neuropharmacology
Branch, NIMH; NINCDS; Columbia University, NIA.

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.8

PROFESSIONAL:

3.3

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Obsessive Compulsive Disorder (OCD) is now recognized as a common mental disorder, occurring in perhaps four million people in this country alone. In adolescents, the prevalence may be one percent.

Positron Emission Tomography with 18 adults with childhood onset OCD shows most significant increase in anterior cingulate and right prefrontal areas. This increase correlates with anxiety state during scan, severity of OCD, and clinical response to clomipramine.

Fifty-one children and adolescents are enrolled in an ongoing double-blind comparison of clomipramine (CMI) and desmethylinipramine (DMI). DMI is virtually without benefit, while 70% of OC subjects receive at least some help from CMI.

Trichotillomania (hair pulling) also appears to respond to CMI. Two single-blind and seven double-blind comparisons with DMI have been completed and results are more dramatic than for OCD.

Adolescents with Sydenham's chorea have significantly higher incidence of OCD than controls with rheumatic fever alone. This implicates the basal ganglia in pathophysiology of OCD.

Project Description:

Objectives: To examine the biological correlates (brain imaging patterns, family psychopathology, cerebrospinal fluid (CSF) chemistry), treatment response, and natural history of obsessive compulsive disorder in childhood and adolescence.

Methods Employed: Resting state Positron Emission Tomography is being compared for adults with childhood onset OCD and age/sex matched controls. Rescan on CMI for responders and non-responders is planned.

A double-blind comparison of clomipramine and desmethylinipramine is ongoing. Baseline CSF is obtained for monoamines and metabolites as well as ACTH, cortisol, dynorphin and basopressin. Fifty-one subjects are in the trial to date. As part of this study, all first degree relatives are interviewed personally, including all siblings age six and over.

Expressed emotion (EE) is being measured in parents of children with OCD. Determinants of EE are being examined as well as the relationship between EE and follow-up status.

A comparison of OC symptoms in adolescents with Sydenham's chorea and Rheumatic fever matched for age and attending the same hospital clinic has been completed. Subjects and their parents complete the Leyton Obsessional Inventory - Child Version. Those with high scores are personally interviewed.

A two-year follow-up has completed of all identified cases of OCD, OCP and other psychiatric disorders with OC features which were found during a community survey of over 5,000 adolescents during 1985-86. Structured interviews for both OCD, OCP, and the SADS were utilized.

A follow-up and CMI maintenance discontinuation study is underway to follow both the history of the disorder and continued usefulness of CMI.

Pilot studies with other aberrant "self grooming" behaviors, such as onychophagia (nail biting) and trichotillomania (hair pulling), are underway. Subjects undergo psychiatric and neuropsychological examination. Trichotillomanics also are studied, using PET, and all first degree relatives are interviewed.

Major Findings: Resting state PET shows significant increase in the anterior cingulate and right prefrontal areas. This increase correlates with state anxiety during the scan, clinical severity of the OCD, as well as clinical response to CMI. Taken together, this suggests that the elevated values probably represent some important aspect of the pathophysiological process in OCD rather than simply an artifact of subjective distress.

The results for 40 of the subjects participating in the double-blind comparison between CMI and DMI have been completed. There were 2 drop outs. Serum plasma concentration does not correlate with oral dose or with clinical response. There is a dramatic decrease in OC symptoms with CMI. DMI has virtually no effect as the data during this phase can be almost superimposed on the placebo response during a previous double-blind comparison with 21 subjects in which placebo and CMI were compared.

Family studies have been completed for 46 probands with 98% of parents and 95% of siblings interviewed personally. A total of 10% of parents, 20% of fathers and 9% of mothers, receive a diagnosis of OCD. Another 10% of parents are considered to have OC personality disorder, while 13% exhibit some OC features that are not clinically diagnosable.

Adolescents with Sydenham's chorea have a significantly higher rate (20%) than do controls with rheumatic carditis alone (0%). This suggests that the basal ganglia are important in mediating the pathophysiology of OCD, at least for some cases.

Pilot data from 10 trichotillomanics who have completed the double-blind CMI-DMI comparison indicate an even more dramatic and specific response to CMI for trichotillomania than for OCD. This data together with PET and Sydenham's data indicate that there may be abnormality of a frontal lobe basal ganglia circuit in OCD in which instinctual behaviors reminiscent of fixed action patterns seen in lower species are inappropriately released.

Significance to Mental Health Research: Obsessive compulsive disorder is now thought to affect four million persons in this country alone. Most have never been treated and are unaware that they have a diagnosable, let alone treatable, disorder. There has been virtually no research on this disabling disorder.

It now appears that research from this center, among others, will not only demonstrate a specific and highly effective

pharmacological treatment, but may reorganize our basic thinking about a number of other disorders which, until the present time, were never related to OCD. Specifically, the association of OC symptoms with Tourettes Syndrome, and Sydenham's chorea suggests basal ganglia dysfunction underlying the syndrome. The response of hair pulling and probably nail biting to CMI indicates that there may be a spectrum of instinctual behaviors related to grooming, safety, and possible sexual activity that can be conceived as belonging to an OCD "spectrum."

Proposed Course of Project: A follow-up study CMI maintenance study is underway. The brain imaging studies will be extended, including volumetric MRI of the caudate. A trial of fluvoxamine for CMI nonresponders has been planned as has a family study of subjects with trichotillomania.

Treatment trials of kleptomania, cross dressing, and other compulsive sexual activities are planned.

Publications

Leonard HL, Rapoport JL. Relief of obsessive-compulsive symptoms by LSD and psilocin in 17-year old obsessive-compulsive boy. Am J Psych 1987;144:1239-40.

Leonard HL, Swedo SE, Rapoport JL, Lenane M, Cheslow D. Treatment of childhood obsessive-compulsive disorder with clomipramine and desmethyylimipramine: a double-blind crossover comparison. Psychopharm Bull, 1988;24:93-5.

Leonard HL. Childhood rituals and superstitions. Developmental and cultural perspective. In: Rapoport JL, ed. Obsessive compulsive disorder in children and adolescents. Washington, D.C.: American Psychiatric Press, Inc. (in press).

Leonard, HL, Rapoport, JL: Drug treatment of children and adolescents with OCD. In: Rapoport JL, ed. Obsessive compulsive disorder in children and adolescents. Washington D.C.: Psychiatric Clinics of North America, in press.

Leonard HL, Rapoport JL. Anxiety disorders in childhood and adolescence. In: Tasman A, ed. American Psychiatric Association Annual Review (Vol. 8), Washington D.C.: American Psychiatric Press, Inc., in press.

Luxenberg JS, Swedo SE, Friedland RP, Rapoport JL, Rapoport SI. Neuroanatomic abnormalities in obsessive compulsive disorder detected with quantitative x-ray computed tomography. Amer J Psychiatry, in press.

Swedo SE. Rituals and releasers: An ethological model of obsessive compulsive disorder. In: Rapoport JL, ed. Obsessive compulsive disorder in children and adolescents. Washington D.C.: APPI Press, in press.

Swedo SE, Rapoport JL. Phenomenology and differential diagnosis of obsessive compulsive disorder in children and adolescents. In: Rapoport JL, ed. Obsessive compulsive disorder in children and adolescents. Washington D.C.: APPI Press, in press.

Swedo SE, Rapoport JL. Biochemistry of obsessive compulsive disorder in childhood. In: Deutsch SI, Application of basic neuroscience to child psychiatry. New York: Plenum Pub. Corp., in press.

Rapoport J. The boy who couldn't stop washing. New York: E.P. Dutton, in press.

Berg C, Whitaker A, Davies M, Flament M, Rapoport J. The survey form of the Leyton Obsessional Inventory - child version: norms from an epidemiological study. J Amer Acad Child and Adol Psychiatry, in press.

Flament M, Whitaker A, Rapoport J, Davies M, Berg C, Kalikow K, Sceery W, Shaffer D. Obsessive compulsive disorder in adolescence: an epidemiological study. J Amer Acad Child and Adolescent Psychiatry, in press.

Wise S, Rapoport J. Obsessive compulsive disorder: is it basal ganglia dysfunction. Amer J Psychiatry, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00178-07
CHP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Structure & Function in Developmental Neuropsych. Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith M. Rumsey, Ph.D., Staff Fellow, CHP, NIMH

Richard Coppola, Ph.D., CBDB, NIMH

Robert Cohen, M.D., Chief, Sect. on Clin. Brain Imaging, LCM, NIMH

Alan Zametkin, M.D., Sect. on Clin. Brain Imaging, LCM, NIMH

Connie Duncan, Ph.D., LPP, NIMH

Edward Fitzgibbon, M.D., LSR, NEI

Michael Goldberg, M.D., LSR, NEI

COOPERATING UNITS (if any)

Clinical Brain Disorders Branch, NIMH; Sect. on Clin. Brain Imaging, LCM, NIMH; Lab. of Psychol & Psychopathology, NIMH; Lab. of Sensorimotor Research, NEI

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.1

PROFESSIONAL:

.20

OTHER:

1.80

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

EEG spectral data shows a differential anteroposterior distribution of theta bilaterally in dyslexics relative to controls when performing a difficult design recognition task. These results suggest differences in activation. These results may be interpreted as less efficient neural processing not limited to left posterior regions nor to verbal tasks in dyslexia.

Nine auditory activation tasks designed for use with PET ⁰¹⁵ blood flow studies have been proposed. Pilot testing of these tasks in normal controls during PET have just begun. Adults with attention deficit disorder, residual type (ADD-RT), studied by Drs. Zametkin and Cohen, show focal frontal decreases in glucose utilization when studied with PET. Neuropsychological evaluations show that these patients show selective deficits in distractibility and in card sorting tasks sensitive to frontal lobe dysfunction. Relationships between metabolic and neuropsychological measures are being examined.

Event-related potential data and eye movement data collected on severely dyslexic men and on "subjective poor readers," respectively, are being analyzed.

Objectives: The primary goal of this project is to identify neuroanatomical, neurophysiological, and neuropsychological abnormalities in developmental dyslexia and in infantile autism. A secondary goal is to determine the utility of various imaging techniques in defining such abnormalities.

Methods Employed: Methods used include PET using ^{15}O -labeled water to study regional cerebral blood flow under various task conditions and fluorodeoxyglucose to study glucose metabolism, magnetic resonance imaging (MRI) to study brain anatomy, spectral analysis and topographic mapping of ongoing electroencephalograms (EEG), event-related potentials, infrared oculography for recording eye movements, neuropsychological testing and behavioral questionnaires.

Major Findings: EEG spectral data shows differences between dyslexic men and their controls in the anteroposterior distribution of theta during a difficult design recognition task. With performance controlled, the dyslexics showed relatively increased frontal and central theta and decreased posterior theta. These differences reflect differences in activation in the dyslexics and the controls. This may reflect inefficiency of neural processing not limited to verbal tasks nor to left posterior regions in dyslexia. This phenomenon may be secondary to more lateralized primary neuropathology or may signal the operation of compensatory mechanisms that recruit increasingly widespread neuronal pools.

The failure to demonstrate lateralized patterns of abnormal cortical activity may stem from the limited sensitivity of electroencephalographic measures to left-right differences. Two contrasting tasks, a word- and a design-recognition task, matched for structural characteristics, failed to produce differentially lateralized patterns of activation. Activation was apparent along an anteroposterior axis with an apparent difficulty effect observed here. The design recognition task proved to be significantly more difficult for both dyslexics and controls than did the word recognition task and resulted in greater posterior alpha desynchronization.

Other data still under analysis includes event-related potential data collected on 15 severely dyslexic men and their matched controls and eye movement data collected on small numbers of slow readers, ADD-RT adults, and controls. Data has been extracted and quantified for both of these studies and is awaiting statistical

analysis. In the case of eye movement data, regressive eye movements while reading appear to distinguish "subjective poor readers," who complain of slow reading, but fail to perform poorly even on more difficult, higher-level standardized reading measures. Thus, eye movements provide a highly sensitive measure of reading efficiency.

Adults with ADD-RT, studied by Drs. Zametkin and Cohen, show focal frontal decreases in glucose utilization when studied with positron emission tomography. A large sample (approximately 30-35) of these adults have been evaluated with a limited battery of neuropsychological test measures. As a group, these patients show selective deficits on the Distractibility factor of the Wechsler Adult Intelligence Scale-Revised (WAIS-R). Approximately 25% of them also show deficits on the Wisconsin Card Sorting Test, which is sensitive to dysfunction of the dorsolateral prefrontal cortex. Approximately six have a history and/or current evidence of an associated learning disability involving reading and spelling. One apparent sex difference consists of an associated math deficit in females, evidenced by significant sex differences on both the Wide Range Achievement Test and WAIS-R Arithmetic subtest. While these phenotypes of adults with familial ADD are of interest in themselves, their ability to account for the variability in metabolic data will be of even greater interest. We are beginning to examine behavioral-metabolic relationships with correlational and subtyping techniques in collaboration with the Section on Clinical Brain Imaging, LCM, NIMH.

Nine tasks have been developed and audiotaped for our PET scan activation studies with 0¹⁵. These have been piloted behaviorally to establish a good level of task difficulty and to select individual items. Our preliminary tapes have been edited and final audiotapes completed. Special audio equipment for presenting tasks during PET has also been developed. We have just begun piloting our tasks physiologically (during PET with 0¹⁵) in carefully screened normal male controls.

Proposed Course of Project:

We are recruiting additional controls and dyslexic and high-functioning autistic patients for our PET studies. Neuropsychological studies, involving right hemisphere and frontal lobe measures, are also planned, particularly for high-functioning autistic patients.

We will also soon begin brain anatomical studies of dyslexic and high-functioning autistic patients with MRI. Our protocol is in the late design phases. Features of special interest are the temporal lobes and their symmetry, the corpus callosum and the cerebellum. We hope to determine the reliability of measures of interest in normals prior to scanning patients and will do this with repeated measures (rescanning normal controls).

Publications:

Rumsey JM, Coppola R, Denckla MB, Hamburger SD. EEG spectra in severe and persistent developmental dyslexia: rest and word and design recognition. In preparation.

Horwitz B, Rumsey J, Grady C, Rapoport SI. The cerebral metabolic landscape in autism: intercorrelations of regional glucose utilization. Archives of Neurology, in press.

Rumsey J, Hamburger S. Neuropsychological findings in high-functioning men with infantile autism, residual state. Journal of Clinical and Experimental Neuropsychology, 1988, 10:201-21.

Rumsey J, Creasey H, Stepanek J, Dorwart R, Patronas N, Hamburger SD, Duara R. Hemispheric asymmetries, fourth ventricular size, and cerebellar morphology in autism. Journal of Autism and Developmental Disorders, 1988, 18:127-37.

Rumsey J, Berman K, Denckla M, Hamburger S, Kruesi M, Weinberger D. Regional cerebral blood flow in severe developmental dyslexia. Archives of Neurology, 1987, 44:1144-50.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00301-06 CHP
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Diagnosis in Child Psychiatry		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Judith L. Rapoport, M.D., Chief, CHP, NIMH Breck Borcharding, M.D., Medical Staff Fellow, CHP, NIMH Eric Taylor, M.D., Sr. Registrar, Maudsley Hospital, London, U.K. James Swanson, Ph.D., Prof., Psychol., Univ. of CA, Irving, CA Michael Rutter, M.D., Prof., Psychology, UCLA, Los Angeles, CA Michael Goldstein, Ph.D., Prof., Psychol., UCLA, Los Angeles, CA		
COOPERATING UNITS (if any) Dept. of Psychiatry, Maudsley Hospital, London, U.K. Dept. of Psychol, University of California, Los Angeles, CA		
LAB/BRANCH Child Psychiatry Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: .20	PROFESSIONAL: .10	OTHER: .10
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The validity of several DSM-III diagnoses remains controversial. Studies are underway across several centers addressing the validity of the diagnostic distinction between situational and cross situational hyperactivity, and between obsessive compulsive disorder (OCD), subclinical OCD, and compulsive personality disorder.</p> <p>In the U.K., great emphasis is placed upon cross situational hyperactivity (based on parent and teacher behavior ratings). This distinction will be validated using 24-hour motor activity measure for hyperactive children and controls in London and Washington.</p> <p>The validity of the distinction between OCD, subclinical OCD, OCP is being assessed with: studies of interrater reliability, associated features and family studies, and follow-up status.</p>		

Objectives: To compare the 24-hour activity of childhood hyperactivity in London, England, and Bethesda, Maryland, for subjects rated hyperactive at home and at school, and for subjects rated hyperactive in only one of these settings. The purpose of the study is to see whether motor activity is best predicted by the teacher rating (at is generally assumed in the U.S.) or whether parent rating accurately reflects pathological elevation, if any.

A second study is examining the reliability and validity of the diagnoses of obsessive compulsive disorder, compulsive personality disorder, subclinical OCD, and other disorders with some OC features, in several samples. Populations include: subjects participating in drug treatment and follow-up studies at the NIH, first degree relatives of these subjects who have received one or more of the above diagnoses, and subjects from an epidemiological study of OCD in adolescents.

Methods Employed: Approximately 20 hyperactive boys, 10 "situational" and 10 "cross-situational" will be studied in each setting using teacher and parent ratings on standardized behavior rating scales, and 24-hour motor activity recordings.

For children and adolescents and their first degree relatives with Obsessive Compulsive Disorder, Compulsive Personality Disorder and subclinical manifestations of obsessive or compulsive traits, interrater reliability, associated features and follow-up status is being assessed.

Major Findings: A major cross national effect of clinician training and diagnostic scheme has been found for the diagnosis of Attention Deficit Disorder between the U.S. and U.K. British clinicians are likely to diagnosis children as Conduct Disordered that would receive the diagnosis of Attention Deficit Disorder in the U.S. The cross national actometer study is still in the planning state and there are no findings on the validity of situationality to date.

With respect to Obsessive compulsive disorder, the NIMH team achieves excellent reliability for the diagnosis of OCD ($\kappa = .85$), but only moderate reliability for subclinical OCD ($\kappa = .54$) and very poor reliability for OCP. In spite of this range in interrater reliabilities (based on typed case vignettes), there is evidence from the follow-up study of these subgroups from a community sample, that there is valid distinction between the groups. Subjects rated subclinical or

OCP tended to do well on two-year follow-up, while subjects having OCD tended to remain ill.

Significance to Mental Health Research: Clinical research in child psychiatry has been hampered by the lack of reliable and valid diagnostic distinctions, both between conduct disorder and for the anxiety disorders. These studies are basic to future clinical and epidemiological research studies in childhood psychopathology.

Proposed Course of Project: Follow-up and family data are being gathered on the clinical cases with obsessive compulsive disorder. Actometer data will be collected over the course of the next year.

Publications:

Taylor E, Rapoport J. Diagnosis of hyperactivity - U.S. and U.K. differences. In: Sargeant J, Bloomingdale L. (Eds.): Research Diagnostic Criteria for Attention Deficit Disorder. New York: Spectrum Publishers, in press.

Rapoport JL. DSM III-R and child diagnosis. In: Last C, Hersen M. (Eds.): Issues in Diagnostic Research. New York: Academic Press, 1987;329-44.

Prendergast M, Taylor E, Rapoport J, Bartko J, Donnelly M, Zametkin A, Ahearn M, Dunn G. The diagnosis of childhood hyperactivity: a U.S.-U.K. cross-national study of DSM III and ICD-9. J Child Psychol, Psychiat, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02240-04
CHP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Attention of Deficit Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith L. Rapoport, M.D., Chief, CHP, NIMH
Markus J.P. Kruesi, M.D., Staff Psychiatrist, CHP, NIMH
William Z. Potter, M.D., Ph.D., Chief, Sect. on Clinical Pharmacol.
LCS, NIMH
Markku Linnoila, M.D, Ph.D., Chief, LCS, NIAAA
Breck Borcharding, M.D., Medical Staff Fellow, CHP, NIMH
Thomas Cooper, M.A., Nathan Kline Institute

COOPERATING UNITS (if any)

Sect. on Clinical Pharmacology, LCS, NIMH
National Institute on Alcohol, Abuse and Alcoholism
Sect. on CLinical Brain Imaging, LCM, NIMH

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.33

PROFESSIONAL:

2.33

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The relationships between behavioral dimensions of aggression, impulsivity, and concentrations of monoamine metabolites, particularly 5-HIAA, in the spinal fluid of children with disruptive behavior disorders of childhood are being examined. As part of that investigation, methodologic variables in spinal fluid studies of children are being clarified. Measurement of socio-environmental variables is being carried out to assess the relations between family environment and serotonergic measures. Follow-up study to address the predictive validity of measurement of CSF 5-HIAA in children and adolescents is underway.

To investigate the role of serotonin in the pathophysiology of ADD, 20 hyperactive boys received fenfluramine (0.6-2.0 mg/kg), dextroamphetamine or placebo in a double-blind crossover study. Fenfluramine had no behavioral effect whatsoever. However, striking biochemical changes (decreased urinary and plasma MHPG, decreased urinary NE, E and VMA). Central serotonergic effects were inferred from the increased plasma prolactin and decreased platelet serotonin. In spite of its structural similarity to amphetamine, fenfluramine has virtually none of its therapeutic effects.

Objectives: 1. To study the pathophysiology of disruptive behavior disorders. 2. To document the utility of CSF studies in childhood mental disorders. 3. To assess interrelationships between socioenvironmental variables and biological variables in disruptive behavior disorders.

Methods Employed: Spinal fluid monoamine metabolites are being obtained from children with conduct disorder, attention-deficit hyperactivity disorder, children with obsessive compulsive disorder as well as a pediatric contrast group. Platelet serotonin, as well as plasma catecholamines, are being collected from the psychiatric subjects. Measures include ratings of impulsivity, aggression, anxiety, depression, and obsessionality.

Socio-environmental variables are being assessed within the families of children with disruptive behavior disorders. Measures include ratings of impulsivity, aggression, anxiety, depression, and obsessionality.

Socio-environmental variables are being assessed within the families of children with disruptive behavior disorders. Measures include assessments of expressed emotion in family members, marital adjustment, hostility, and psychiatric family history.

Major Findings: In a study comparing 25 hyperactive boys and their matched controls, the hyperactive boys demonstrated significantly more difficulties in cognitive tasks which required more effortful (i.e. conscious and active) processing than did the control boys. The groups did equally well at tasks which were more automatic and rote in nature. This study provides a different focus on the concept of attention, and supports notion that motivation, affect, and other higher order cognitive processes may be important variables in remediating the effortful deficit.

Preliminary results of a double-blind, placebo controlled crossover trial of methylphenidate and dextroamphetamine in 18 hyperactive boys reveals that methylphenidate lowers truncal activity counts of these boys in a day hospital setting more than dextroamphetamine at all dose ranges studied. Currently, these results are being analyzed in concert with stimulant blood level data and urinary and plasma catecholamine values.

Concentration gradients of the monoamine metabolites, homovanillic acid and 5-hydroxyindolacetic acid are present in

the lumbar spinal fluid of children and adolescents. The slopes of these gradients are such that failure to control for the aliquot collected can create suprious findings.

Side effect data on lumbar puncture in children and adolescents has been rare and confounded by the administration of agents into the spinal canal or by the use of general anesthesia. We have prospectively collected data on the incidence of side effects of research lumbar punctures as well as information on the subjective experience from the child's perspective. Half of the children and adolescents surveyed indicated attending school was more noxious than their LP.

Comparison of ten age-matched conduct disorder and obsessive compulsive disorder children showed a trend ($p = .09$) for lower concentrations of 5-HIAA and Brown-Goodwin Scale ratings of aggressive acts.

We found high rates of high expressed emotionality in parents of children with disruptive behavior disorder. Fathers of disruptive behavior disorder children who have high expressed emotion are significantly more likely to have a psychiatric diagnosis than those with low expressed emotion.

Proposed Course of Project: Following collection of CSF from two more subjects data will be analyzed. Cross sectional examination of relationships between the behavioral dimensions of impulsivity and aggression with concentrations of CSF monoamines will be carried out. Recruitment will continue in order to enlarge subgroups, particularly those who have cruelty to animals. Follow-up study is underway to see if CSF 5-HIAA concentration predicts follow-up status. The relative impact of social factors and biochemistry on subsequent status will be essential.

Significance to Mental Health Research: Conduct disorder is the most frequent cause for referral of children and adolescents for psychiatric services. The preliminary trend for lower 5-HIAA concentration in the CSF of conduct disorder subjects is consistent with studies in adults relating impulsive and aggressive behavior with decreased 5-HIAA, and HVA, establishes the need for methodological control over the aliquot collected a point not addressed in previously in pediatric behavioral studies.

The data presented in this paper concerning children's responses to lumbar puncture is very important in establishing standards

that can be used to make informed judgments concerning risk benefit considerations for research purposes. The authors are making a real contribution to the establishment of research procedures. Systematic assessment of the side effects of a procedure that is viewed with alarm is extremely useful.

The evidence that sugar challenge does not alter aggression has been great interest to others, as evidenced by the large number of reprint requests.

Publications

Kruesi MJP, Rapoport JL, Berg C, Stables G, Bou E. 7-day carbohydrate and other nutrient intakes of preschool boys alleged to be behavior responsive to sugar intake and their peers. In: Essman WB, ed. Nutrients and brain function. Basel: Karger, 1987;133-7.

Kruesi MJP, Rapoport JL, Cummings EM, Berg C, Ismond DI, Flament M, Yarrow M, Zahn-Waxler C. Effects of sugar and aspartame on aggression and activity in children. Amer J Psychiatry, 1987;144:1487-90.

Kruesi MJP, Rapoport JL. Aspartame and children's behavior. In: Williams GM, ed. Sweeteners: health effects. Princeton, NJ: Princeton Scientific Publishing Co., 1988;173-8.

Borcherding B, Thompson K, Kruesi MJP, Rapoport JL, Weingarter H. Automatic and effortful processing in attention deficit/hyperactivity disorder. J Abnormal Child Psychol, 1988;16:333-45.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00084-14 CNG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Genetic-Biologic Studies of Psychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	E.S. Gershon	Chief	CNG, NIMH
Others:	L.R. Goldin	Res. Geneticist	CNG, NIMH
	M. Martinez	Guest Researcher	CNG, NIMH
	D. Jimerson	Psychiatrist	Harvard Univ.
	J. Kassett	Res. Social Worker	LCS, NIMH
	M.E. Maxwell	Res. Social Worker	CNG, NIMH
	J. Hamovit	Res. Social Worker	CNG, NIMH

COOPERATING UNITS (if any)

LCS, DIRP, NIMH
Harvard University

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20892

TOTAL MAN-YEARS:

7 3/4

PROFESSIONAL:

3

OTHER:

4 3/4

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

1. Analytic methods in clinical genetic studies. a) Statistical power of current methods to detect linkage and heterogeneity, when penetrance is less than one (as appears present in the linked psychiatric disorders): Linkage can be detected with very feasible sample sizes of small families in the presence of considerable heterogeneity. When flanking markers are available, both linkage and heterogeneity are more readily detectable. b) Association methods. These are useful in attempting to identify candidate causative genes within a linkage region, through association with illness due to linkage disequilibrium. Analysis of the power of detection of disequilibrium in the presence of heterogeneity reveals that for several realistic parameter sets, only one or two linked pedigrees are needed.

2. Pedigree collection for manic-depressive illness and schizophrenia molecular mapping studies continues. In manic-depressive illness, we have 15 pedigrees with more than 200 individuals in culture. In schizophrenia, we have 7 pedigrees with more than 70 individuals in culture.

3. Family study of bulimia has been completed, supporting the co-aggregation in families of anorexia and bulimia suggested in earlier studies of anorexia, and demonstrating the co-aggregation of affective disorders with bulimia.

Others:	A. Smith	Res. Social Worker	CNG, NIMH
	W.H. Berrettini	Medical Officer	CNG, NIMH
	J. Gelernter	Med. Staff Fellow	CNG, NIMH

I. Current progress

A. Family Study of Bulimia

1. Bulimia Family Study

The prevalence of psychiatric disorders, diagnosed by modified RDC criteria, was studied in relatives of 40 bulimic patients (who had not had anorexia), and compared with the prevalence of these disorders in a relatives of 24 normal controls. Relatives of probands with bulimia had significantly increased eating disorders (anorexia and bulimia), major affective disorder (bipolar plus unipolar), alcoholism, and a non-significant trend toward increased drug abuse (Table). These results are non-hierarchical; that is, a relative can have and be counted for more than one diagnosis.

These results, taken together with our previous similar results in relatives of patients with anorexia, imply that a common familial basis for eating disorders and affective disorders is present in families in which both types of disorders appear. The implication for genetic linkage studies is that mood disorders (but not in schizophrenia) relatives with bulimia (and anorexia) should be considered as affected. The implication for prevention and treatment is that efforts to deal with eating disorders should bear in mind the co-aggregation and co-morbidity with affective disorders.

Table: Morbid risk of selected diagnoses in adult first-degree relatives of bulimics

Relatives' morbid risk (%)				
	<u>Bipolar + Unipolar</u>	<u>Anorexia + Bulimia</u>	<u>Alcoholism</u>	<u>N</u>
Proband diagnosis				
Bulimia	27.9***	11.8*	27.6**	85
Control	8.8	3.5	13.6	118
p<.05	**p<.01	***p<.001		

B. Population Genetics Methods

1. Power of linkage analyses for heterogeneous disorders

We have previously examined the power of the affected-sib-pair method and of the lod score method in moderate sized pedigrees to detect linkage in the presence of heterogeneity and complex inheritance. We have extended these studies to examine the required sample size of nuclear families to detect both linkage and heterogeneity.

We have considered this problem in a very general way, allowing for phase unknown offspring and variation in the genetic model parameters in order to allow for decreased penetrance and phenocopies. We have studied the case of close linkage (recombination = .01), 50% of families linked, and fully informative markers. We have varied the family size and the sampling strategy. The sample sizes determined are those needed for a significant ($\alpha=.05$) chi-square likelihood ratio test for: 1) linkage vs. no linkage and 2) heterogeneity vs. homogeneity and were found by exact likelihood calculations.

In the case of recessive inheritance, sample sizes needed to detect linkage or heterogeneity are feasible. For example, with high penetrance and sampling of families with 2 sibs with at least 1 affected, 32 families are needed to detect linkage and 377 needed to detect heterogeneity. However, if we select families having 3 sibs, of which at least 2 are affected, then 8 families are needed to detect linkage and 71 needed to detect heterogeneity. For the case of dominant inheritance, the numbers of families needed to detect linkage are similar but heterogeneity is almost impossible to detect. For example, with high penetrance, and families of 3 sibs with at least 2 affected, 9 families are needed to detect linkage but 5737 are needed to detect heterogeneity. When the penetrance is decreased, over 100,000 are needed. However, with families of 4 offspring and at least 2 affected, only 50 families are needed to detect heterogeneity. We have also considered the increased power of flanking markers, assuming that the location of the 2 marker loci are fixed and the disease locus is allowed to vary freely. Consistent with other studies, there is a greatly increased power when an additional marker locus is present. For example, in the recessive case above where 377 families are needed to detect heterogeneity, only 25 families are required if an additional marker locus is present.

2. Expected linkage information in our pedigree sample

At the time these computations were performed, we had 8 moderate sized pedigrees with affective disorders in cell culture. For schizophrenia, we had 3 moderate sized pedigrees and a larger number of affected-sib-pair families. In order to determine how informative these families were for linkage, we calculated the expected lod scores in these families by simulation. Simulations here are based on 1 fully informative marker locus with a recombination frequency of 1% with the disease locus. The disease locus is assumed to be a dominant gene with high penetrance (85%). Under these assumptions, the average expected lod score per pedigree is 1.5 with a range of 0.8 - 2.3. If penetrance is lowered (50%), the average lod score is 0.8 with a range of 0.3 - 1.3. When there is no linkage, average lod scores are -2.4 and -1.2 for high and low penetrance, respectively. Thus it is clear that our families are very informative for detecting

or rejecting linkage although none of them alone will give a lod score of +3.0. This sample is powerful even for some heterogeneity. Our previous calculations indicate that with 10 families of the size we are studying, we have 70% power to detect close linkage if only 25% of the families are linked.

3. Detection of the causative disease mutation in a linked illness

Once linkage is detected, the causative gene must be identified. Bodmer has pointed out that in this search, finding linkage disequilibrium through population association may prove more efficient than trying to identify more closely linked markers. We have analyzed the effects of heterogeneity on detection of a disease association, and demonstrated that, using new sampling methods, association can be detected with smaller samples than was previously thought possible. For example, two linked pedigrees provide 80% power to detect an allele whose frequency on chromosomes with a disease mutation is 0.6, but on normal chromosomes is 0.01%.

C. Pedigree collection

1. Manic depressive illness

A total of 15 pedigrees of bipolar probands have been collected, including approximately 100 ill subjects and 200 well subjects. Final best-estimate diagnoses and cultured lymphoblast cell lines have been established for approximately 75% of these subjects.

2. Schizophrenia

A total of seven pedigrees of schizophrenic probands have been collected, including approximately 35 ill subjects and 75 well subjects. Final best-estimate diagnoses and cultured lymphoblast cell lines have been established for approximately 75% of these subjects.

D. Family/pedigree methods course

A four day course was offered by the Clinical Neurogenetics Branch, under the direction of Mrs. M. Elizabeth Maxwell and Dr. Elliot Gershon, in July of 1987 and is planned to be offered again in July of 1988. Topics covered were design and analysis of family/pedigree studies, systematic interview methods, systematic diagnosis methods and standardization, establishment of diagnostic reliability, data organization and records keeping, and role of genetic linkage and mapping in investigation of psychiatric disorders. Twenty-four participants attended, including participants from the University of Utah, University of Tel Aviv (Israel), and intramural participants. This course has generated collaborations among participants, since diagnostic methods and reliability are established during the course, thus facilitating combining of data and linkage scores between pedigrees.

II. Significance To Biomedical Research and the Program of the Institute and Proposed Course of Study

Determination of an appropriate number and type of pedigrees suitable for psychiatric linkage studies is a bottleneck to further genetic progress in these disorders. By suitable analyses and simulations, we are establishing the types of and number of pedigrees needed for the conditions of heterogeneity and reduced penetrance present in the major psychiatric disorders. We are collecting the required pedigrees. Through our methods course, we are generating appropriate collaborations so that all pedigrees have standardized data collection.

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PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Outpatient Clinic for Genetic and Pharmacologic Studies of Affective Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Wade H. Berrettini	Medical Officer	CNG, NIMH
Others:	E.S. Gershon	Chief	CNG, NIMH
	S. Simmons-Alling	Clinical Nurse Expert	CC, NIH
	M.E. Maxwell	Res. Social Worker	CNG, NIMH
	A.L. Smith	Res. Social Worker	CNG, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4 3/4

PROFESSIONAL:

3

OTHER:

1 3/4

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

A longitudinal prospective study of adolescents at high risk for affective disorder is continuing. A biological marker, the suppression of plasma melatonin by light, differentiates the "high-risk" adolescents from a control adolescent group.

CSF dynorphin A₁₋₈ immunoreactivity is significantly elevated in Tourette's Syndrome compared to age and sex matched controls. The degree of elevation is significantly correlated with severity of obsessive-compulsive behavior (but not with severity of motor tics), suggesting that this peptide may play a role in pathophysiology of this disorder.

Post-mortem ventricular fluid (PMVF) was obtained at autopsy from subjects with Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD) and controls dying of cause not related to the CNS. Measurements of galanin, neuropeptide Y, and somatostatin revealed that all were significantly elevated in every patient group compared to the controls, findings that are contrary to in vivo lumbar CSF studies, suggesting that PMVF may not be a valid approach to the study of neuropeptides in disease states.

Investigators Continued:

J.F. Leckman	Assoc. Prof. of Psychiatry	Yale Univ.
M.A. Riddle	Asst. Prof. of Psychiatry	Yale Univ.
J. Gelernter	Med. Staff Fellow	CNG, NIMH
L. Goldin	Res. Geneticist	CNG, NIMH
W.H. Kaye	Assoc. Prof. of Psychiatry	Univ. of Pitts.
D. Pellegrini	Asst. Prof. of Psychiatry	Catholic Univ.
T. Sunderland	Staff Fellow	LCS, NIMH
M. Linnoila	Clin. Director	NIAAA
P. Gold	Chief	SCN, BPB, NIMH
C. May	Sr. Staff Fellow	LNS, NIA
L. Tamarkin	Clin. Assoc.	CPB, NIMH
D. Rubinow	Clinical Director	DIRP, NIMH
G. Oxenstierna	Staff Psychiatrist	Karolinska Inst.
G. Sedvall	Prof. of Psych.	Karolinska Inst.
N. Cutler	Staff Psychiatrist	UCLA

PROJECT DESCRIPTION:

We maintain an outpatient clinic of approximately 100 bipolar subjects for the purpose of: 1) identifying potential markers of genetic vulnerability to affective disorder; and 2) studying the onset and course of illness of bipolar disease. In addition to the bipolar subjects themselves, we are conducting a study of the healthy adolescent children of bipolar parents, children at "high risk" for the development of affective disorder.

We study euthymic (well-state) bipolar subjects to determine those abnormalities which are most likely to be inherited characteristics of the illness. Additionally, the study of children at high risk for affective illness is useful in avoiding drug effects or secondary effects of the illness which may persist even in the euthymic state.

For comparison purposes we maintain a pool of approximately 50 well-screened normal volunteers.

Lastly, this project includes an active laboratory devoted to the study of CSF neuropeptides in psychiatric diseases. This laboratory has collaborated with clinical investigators, attempting to elucidate the roles of various neuropeptides in eating disorders, affective illness, Alzheimer's disease and schizophrenia.

Each aspect of the project is described below.

1. High Risk Study

We are conducting a longitudinal prospective study of well adolescent (age 15-25) children (N=82) of bipolar parents with age-matched children (N=56) of control parents. These adolescent children of bipolar parents are being studied prior to onset of affective illness. Thirty percent of them are expected to become ill. By conducting this prospective study, we hope to identify biological or psychological variables that will predict who will become ill. Subjects are interviewed annually for evidence of affective disorder. To date 7 children of bipolar parents have

become ill (1 bipolar and 6 unipolar cases), while one control child has developed affective disorder during the first three years of the study.

One potential vulnerability marker has been identified. Unmedicated bipolar subjects, independent of mood state, show supersensitivity to the inhibitory effects of light on plasma melatonin. We studied these adolescents using this paradigm in collaboration with Dr. Larry Tamarkin. Ten of 23 adolescents of bipolar parents were supersensitive, compared to 3 of 22 controls. This result suggests that the supersensitivity to light may be a genetic vulnerability marker for affective disorder. However, studies on the heritability of this response and segregation studies in pedigrees are necessary to confirm this putative marker.

2. CSF Neuropeptides

Disease-related studies:

We have continued to study CSF neuropeptides in psychiatric disorders, to determine the extent to which CSF levels of a neuropeptide can reflect abnormal behaviors or reveal aspect of pathophysiology. We have developed two new assays for measurement of LHRH and dynorphin A in CSF. In collaboration with James Leckman and Mark Riddle of Yale University, we measured CSF dynorphin A₁₋₈ immunoreactivity in 8 adult subjects with Tourette's Syndrome (TS) and matched controls. CSF dynorphin levels were significantly elevated in the TS patients ($p < .05$) was not seen among TS subjects ($r = -.05$), suggesting that a dynorphin-serotonin relationship might be disturbed in the TS group.

In collaboration with Neal Cutler of UCLA, post-mortem ventricular fluid (PMVF) was obtained from 5 subjects with Parkinson's disease (PD), 5 subjects with Huntington's disease (HD), 13 subjects with Alzheimer's disease (AD) and 14 subjects dying of causes unrelated to CNS function. Neuropeptide Y (NPY), galanin, and somatostatin (SRIF) were determined. Results are shown in the table below:

	CONTROLS	AD	PD	HD
GAL	18.8 +/- 18.6	23.8 +/- 6.4	37.8 +/- 28	41.6 +/- 10
NPY	217 +/- 147	432 +/- 296	606 +/- 316	852 +/- 280
SRIF	61 +/- 37	83 +/- 32	201 +/- 111	302 +/- 361

Values given are in pg/ml, mean +/- standard deviation.

The control SRIF values are similar to those obtained with lumbar CSF in vivo, while both galanin and NPY values are higher than those found in lumbar CSF. The three experimental groups are all elevated, compared to the control group, suggesting that non-specific elevations may occur in the peri-mortem period, as the experimental subjects had prolonged agonal periods compared to the controls. This study suggests that PMVF may not be a valid approach to the assessment of brain neuropeptide function. Assessment of PMVF has the advantage that the subjects have had pathologically-confirmed diagnoses for the CNS disease under study.

Given the hypothesis that noradrenergic hyperactivity occurs during alcohol withdrawal states, several neuropeptides linked to CNS noradrenergic function were measured in the CSF of alcoholics during stages of withdrawal, in collaboration with Markku Linnoila, NIAAA. No significant differences were found for CSF NPY or CSF GHRF compared to controls.

We have previously reported that chronically underweight subjects with anorexia have elevated CSF NPY, a neuropeptide that potently stimulate feeding behavior in animal studies, when given ICV or into the paraventricular nucleus. CSF NPY levels remain elevated in the anorexic subjects even after they have returned to ideal body weight, particularly if they remain amenorrheic. This observation is intriguing because NPY causes release of FSH and LH from the pituitary after intrahypothalamic injection. We have developed an assay for LHRH in CSF. Normal values are approximately 4 pg/ml. The nature of the immunoreactivity has been characterized by HPLC as authentic LHRH. We plan to measure LHRH in the CSF of these anorexic females to determine if there is any relationship between NPY and LHRH in these subjects. We also plan to determine these peptides in CSF from women with premenstrual syndrome and those with secondary idiopathic hypothalamic amenorrhea.

3. Growth Hormone Response to Clonidine

The growth hormone response to clonidine has been reported to be blunted in subjects with affective disorder. To determine whether this is a state-independent phenomenon, we are studying unmedicated euthymic bipolars and controls. To date 18 bipolars and 23 controls have been studied. The bipolars do show a significantly blunted growth hormone response ($p < .04$). We have studied the same euthymic patients during lithium treatment and when unmedicated to assess the effect of lithium on this putative marker. These results are being analyzed. This measure may represent a clinically available test of alpha adrenergic function in affective disorder. Moreover, this test may be a useful method of selecting pedigrees to search for linkage to restriction fragment length polymorphisms of the alpha adrenergic receptor gene, a project which has shown no relationship between a blunted GH response and a D α 1 RFLP in preliminary studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

The study of adolescents at high risk for affective disorder has revealed a putative biological marker for development of illness in the light supersensitivity to melatonin inhibition. Through annual follow-up interviews, we hope to be able to determine the relationship between melatonin inhibition by light and illness vulnerability. The identification of such a marker would have tremendous importance for the focus of genetic research in affective disorders. The annual follow-up interviews will also allow us to delineate the subclinical changes (if any) which precede the development of the first episode of illness. If there are such subclinical changes, their identification would have obvious and immediate importance for early intervention/prevention efforts of the Institute regarding both affective

disorders in general and teenage suicide in particular.

The finding of elevated dynorphin A CSF immunoreactivity in TS subjects is a provocative one, because large amounts of dynorphin have been found in the basal ganglia, an anatomical site which has been suspected as the origin of TS pathophysiology. The positive significant correlation between obsessive compulsive symptom severity and CSF dynorphin levels is consistent with the hypothesis that abnormal dynorphin function in the basal ganglia may be involved in the pathophysiology of TS.

The findings of significant elevations in PMVF neuropeptides across several neuropsychiatric diseases raises interesting questions concerning the significance of PMVF determinations of these peptides. Determination of PMVF levels of 5-HIAA seem to correlate well with brain cortical 5-HIAA measurement, according to the data of Stanley et al., and levels in PMVF are not altered from those obtained *in vivo*. This may not be the case with neuropeptides. For example, every study of lumbar CSF SRIF in AD has reported reduced levels, compared to control values. PMVF SRIF levels seem to be elevated in AD, compared to controls (although not significantly so). Careful assessment of the characteristics of the agonal state and correlation with CSF neuropeptide findings may be needed to clarify the significance of PMVF neuropeptides. The ability to confidently use PMVF as an approach to the study of neuropsychiatric diseases is important to determine, because brain tissue may be difficult to obtain and because the subjects often have pathologically-confirmed diagnoses. Additionally, PMVF may avoid sampling spinal cord contribution to CSF levels of peptides.

PROPOSED COURSE OF STUDY

We propose to continue the high risk study, with annual follow-up interviews. Additionally, we propose to study additional high-risk adolescents as they become available, to expand the number of observations, as only 30% of the high-risk group is expected to become ill.

We propose to expand the study of TS subjects by performing a confirmatory study of a second population of TS subjects. In addition, we hope to develop assays for other dynorphin moieties in CSF, to determine their relationships to TS symptomatology. We hope to explore the role of dynorphin in other "basal ganglia diseases" including progressive supranuclear palsy.

We plan to continue the study of PMVF to determine the clinical antemortem parameters which might have caused the elevations which we have observed.

The continuation of the clonidine study is necessary in order to increase the number of observations, and to correlate the GH response to α_2 -adrenergic receptor gene RFLPs which have been discovered in our laboratory.

Publications

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02236-04 CNG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Schizophrenia Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Dauphinais	NRSA Fellow	CNG, NIMH
Others:	E.S. Gershon	Chief	CNG, NIMH
	L.R. Goldin	Res. Geneticist	CNG, NIMH
	D. Kazuba	Psychologist	CNG, NIMH
	J. Guroff	Soc. Sci. Analyst	CNG, NIMH
	E. Maxwell	Res. Soc. Worker	CNG, NIMH
	L. DeLisi	Psychiatrist	SUNY Stony Brook

COOPERATING UNITS (If any)

SUNY, Stony Brook, NY

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20892

TOTAL MAN-YEARS

4 3/4

PROFESSIONAL:

3

OTHER:

1 3/4

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects
 ☐ (b) Human tissues
 ☐ (c) Neither
- ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Brain structural anatomy in familial schizophrenia was studied with magnetic resonance imaging (MRI). MRI scans of 25 schizophrenic siblings from 14 unrelated families, 8 of their non-psychotic siblings, and 20 controls were analyzed. Volume estimates were made of the cerebrum, temporal lobes, amygdala-hippocampus, amygdala-hippocampus plus parahippocampal gyrus, caudate, lentricular nucleus, lateral ventricles, and third ventricle. Compared with the control sample the schizophrenic siblings had significant volume reduction of the temporal lobes, bilaterally, and significant increase in ventricular brain ratio (VBR). These structural abnormalities may be part of the pathophysiology of the disease.

This project is now complete and will terminate as of September 30, 1988. Further molecular genetic studies of schizophrenia will be reported in other project summaries.

Brain structural anatomy in familial schizophrenia was studied with magnetic resonance imaging (MRI). MRI scans of 25 schizophrenic siblings from 14 unrelated families, 8 of their non-psychotic siblings, and 20 controls were analyzed. Volume estimates were made of the cerebrum, temporal lobes, amygdala-hippocampus, amygdala-hippocampus plus parahippocampal gyrus, caudate, lenticular nucleus, lateral ventricles, and third ventricle. Compared with the control sample the schizophrenic siblings had significant volume reduction of the temporal lobes, bilaterally, and significant increase in ventricular brain ratio (VBR). These structural abnormalities may be part of the pathophysiology of the disease.

	Schizophrenics	Controls	P
Temporal Lobes (cm ²)			
Left	39.3 ± 5.0	42.9 ± 5.9	.030
Right	41.2 ± 4.8	45.6 ± 5.9	.010
Total	80.5 ± 8.4	88.5 ± 1.7	.009
VBR in %			
Left	2.5 ± 1.0	1.6 ± .6	.002
Right	2.3 ± .9	1.7 ± .7	.017
Total	2.4 ± .8	1.6 ± .6	.003

Publications

Goldin LR, DeLisi LE, Gershon ES. Unravelling the relationship between genetic and environmental risk factors in psychiatric disorders. *Br J Psychiatry* 1987;151:302-5.

Goldin LR, DeLisi LE, Gershon ES. Genetic aspects to the biology of schizophrenia. In: Henn FA, DeLisi LE, eds. *Handbook of schizophrenia*, Vol. 2. Amsterdam: Elsevier Science Publishers, 1987;467-487.

DeLisi LE, Dauphinais ID, Goldin LR, Gershon ES. Perinatal risk factors in familial schizophrenia. In: Dunner DL, Gershon ES, Barrett JE, eds. *Relatives at risk for mental disorders*. New York: Raven Press, 1988;267-277.

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DeLisi LE, Buchsbaum MS, Holcomb HH. Increased temporal lobe glucose utilization in schizophrenia. *Biol Psychiatry*, in press.

Buchsbaum MS, DeLisi LE, Holcomb HH, Wu J, Hazlett E, Cohen RM, Langston K, Kessler R. Comparison of neuroleptic drug effects and differences between normal controls and schizophrenic patients with Positron Emission Tomography. *Biol Psychiatry*, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02237-04 CNG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	S. Detera-Wadleigh	Sr. Staff Fellow	CNG, NIMH
Others:	C. deMiguel	Visiting Fellow	CNG, NIMH
	I. Encio	Visiting Fellow	CNG, NIMH
	F. Friedberg	Professor	Howard Univ.
	B. SenGupta	Biologist	Howard Univ.
	D. Kligman	Staff Fellow	NINCDS
	T. Bonner	Sr. Staff Fellow	LCB, NIMH

COOPERATING UNITS (if any)

Howard University
NINCDS

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20892

TOTAL MAN-YEARS:

10 3/4

PROFESSIONAL:

5

OTHER:

5 3/4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Chromosome 11: We have identified an Ashkenazi Jewish pedigree 441, where current lod score with HRAS1/INS is 1.1 (possible linkage). New informative members have been recently located. A newly cloned muscarinic receptor gene (m4) is located in the short arm of 11 by cytogenetic methods. We have identified a polymorphism of m4 with SsrI. Pedigree 441 is informative for this gene, and shows no recombination of m4 with manic-depressive illness or HRAS1/INS, although in combining this with three other pedigrees we have observed 15% recombination with HRAS1/INS. Amish pedigree 110 was not informative on this m4 polymorphism. With an extension of the m4 probe, we have observed a new polymorphism with RsaI, which is segregating in the Amish pedigree.

Chromosome 5: A balanced translocation at 5q11 associated with schizophrenia in one family has been reported. A two-allele polymorphism of the glucocorticoid receptor gene (GRL) in that region, has been reported with BclI. We have studied 3 extended pedigrees with schizophrenia. Using broad diagnostic inclusion criteria, there is a possible linkage. This polymorphism, however, did not distinguish all parental alleles. We have extended the GRL probe using a genomic library, in an attempt to increase the linkage information in the 3 extended pedigrees. Another marker, DHFR, reported to be located on 5q11-13, was not linked to GRL or to schizophrenia in these 3 pedigrees. A genetic map of 5q11-13 is being constructed.

Color blindness region of X-chromosome: Six new affective disorders pedigrees have been examined using the St-14 polymorphic marker on Xq28 with no evidence of linkage.

Neuropharmacological candidate genes: RFLPs were found at beta₁ and beta₂ adrenergic receptor gene regions. Linkage to affective disorders was excluded.

Cloning of genes involved in neurotransmission: 4 clones of protein kinase C substrate (87PKCS) have been isolated from lambda gt11-rat brain cDNA library. Analysis of gene expression by Northern hybridization revealed that one of the cDNAs isolated corresponds to the expected expression of the 87PKCS gene in mouse parent and mutant lymphocyte cell lines. For a second clone, the sequence and pattern of expression is different from the other 2 clones. All clones share sequence homology. A family of substrate proteins may be present.

RFLP resolution: Field inversion gel electrophoresis (FIGE) was found to improve the DNA separation by a factor of 2 for fragments ranging from 23.1 kb to 9.4 kb as well as fragments of 4.4 to 2.0 kb in size.

Others (cont):	J. Patel	Biologist	ICI Co.
	W. Berrettini	Med. Officer	CNG, NIMH
	W.-T. Hsieh	Staff Fellow	CNG, NIMH
	P. Gejman	Visit. Assoc.	CNG, NIMH
	J. Gelernter	Med. Staff Fellow	CNG, NIMH
	M. Hoehe	Guest Res.	CNG, NIMH
	C. Venter	Sr. Staff Fellow	LNP, NINCDS
	U. Lentes	Chemist	Bonn Univ.
	L. Goldin	Geneticist	CNG, NIMH
	L. DeLisi	Psychiatrist	SUNY Stony Brook
	E. Gershon	Chief	CNG, NIMH
	W. McBride	Biologist	NCI

Project description

I. Types of investigation:

A. Clinical investigations:

1. Linkage studies of schizophrenia (SZ) and affective disorders (AD).
2. Mapping of regions in which linkage or cytogenetic abnormalities have been reported in psychiatric disorders (11p15, 5q11-13, Xq28).
3. Candidate gene loci in SZ and AD.

B. Basic investigations in support of clinical investigations:

1. Advancement of gene mapping methodology.
2. Cloning and expression neurobiologically important genes.

II. Major findings

A. Chromosome 5 (5q11-13)

1. Glucocorticoid Receptor Gene.

A frequent biallelic RFLP identified by us as well as by Murray et al (Nuc Acids Res 15,6765, 1987) was used to track the GRL genotypes in 3 pedigrees with SZ. The GRL probe was generated by labelling the released insert of the cDNA clone pOB7, which was kindly provided by R. Evans of the Salk Institute. The gene has been mapped to 5q 11-13 but also shows homology to a sequence on chromosome 16. The polymorphic fragments were at 4.5 kb and 2.5 kb, with a population frequency of 0.55 and 0.45, respectively. When lod scores were calculated under a dominant mode of inheritance model and a wide classification of "affected" (based on family studies) the highest cumulative value was 1.3 at a recombination fraction (theta) of 0.1 (see Table 1). Under more restrictive criteria for the affected category the sum of the lod scores are lower (Table 1). These pedigrees are clinically unusual, because of the mixture of affective and schizophrenic diagnoses. We have also added information on 24 affected sib-pairs with chronic psychosis (schizophrenia or schizoaffective). If we analyze only the affected-sib-pairs in the entire sample, we find that the respective proportions sharing 2, 1, or 0 alleles identical by descent are .28, .53, .19 which is not different from random sharing (i.e. .25, .50, .25). Given the likelihood of

heterogeneity of schizophrenia, it is not surprising that adding a large number of smaller families reduces the evidence for linkage.

Nonetheless, these results are intriguing as well as encouraging. Further analysis of these pedigrees using more polymorphic probes in this region would allow a definitive conclusion to be reached on whether there is linkage in the three extended pedigrees.

To pursue this possibility, genomic clones have been isolated from a human genomic library using 3' untranslated region probes to extend the GRL. This portion of the cDNA maps exclusively to the 5q11-13 region. Fragments which recognize little or no repeats are being used for RFLP search. Larger clones representing longer extensions of GRL are being isolated also from a human genomic cosmid library.

The GRL genotypes in 8 pedigrees with AD including the Amish pedigree 110 were also determined. There is no evidence at all for linkage of GRL to affective disorders.

Table 1: Linkage to Glucocorticoid Receptor Gene of Schizophrenia and Related Disorders in Three Families

Lod Scores	Recombination Fraction					
	0	.05	.10	.20	.30	.40
Model (1)	-3.39	-2.11	-1.22	-.37	-.06	-.003
Model (2)	-1.90	-.46	.20	.53	.40	.13
Model (3)	.37	1.0	1.31	1.15	.70	.26
(1) Schizophrenia, Schizoaffective (2) + Schizotypal, Schizoid (3) + Bipolar, Unipolar						
Genetic parameters: dominant, $q = .01$, penetrances .95, .95, .001						

2. Other Genes on 5q11-13

a. Dihydrofolate reductase (DHFR) Gene

A plasmid subclone of a 0.3 kb DHFR intron was kindly supplied by T. Shimada of the NIH. This genomic fragment was previously found by Shimada et al to map exclusively to 5q11-13 and therefore used for RFLP search. Rsa I

detected a two-allele polymorphism at 1.3 kb and 0.5 kb with allele frequencies of 0.68 and 0.32. Close linkage of schizophrenia to DHFR could be rejected (Lod = -2.0 at 0 = 0.0). However, DHFR does not appear to be closely linked to GRL (Lod = -2.5 at 0 = .01). In order to confirm this finding, additional informative pedigrees are being typed.

b. G-21 gene

The G-21 cDNA probe provided by B. Kobilka of Duke University failed to detect an informative RFLP in the 3 SZ pedigrees under study. In the hunt for information on the G-21 locus a genomic clone with an insert size greater than 40 kb was isolated from a human placental cosmid library. Digestion with EcoRI yielded 7 fragments ranging in size from 0.5 kb to 23 kb. These fragments or portions thereof will be used for RFLP search, examining linkage to SZ and to other markers on 5q11-13.

B. Chromosome 11 (short arm)

This region is of interest because of the reported linkage of INS/HRAS1 markers in this region to manic-depressive illness in Amish pedigree 110.

1. m4 muscarinic acetylcholine receptor gene.

This project is being done in collaboration with T. Bonner of LCB, IRP, NIMH, who provided probes for a family of muscarinic receptor genes and mapped m4 to 11p. A 2.4 kb genomic clone which spans the coding region was found to detect an Sst I RFLP and the allelic fragments were at 3.5 kb and 3.2 kb. Segregation of the alleles was observed only in a few pedigrees examined and not in the Amish pedigree 110. In 4 informative families, there was a suggestion of loose linkage of m4 to INS/HRAS1 (lod score = 1.1 at 15% recombination).

To find a highly polymorphic marker at the m4 locus a fragment from a cosmid clone was tested for RFLP. So far an Rsa I RFLP has been detected. Potentially informative matings have been found within the large Amish pedigree, therefore, the precise mapping of m4 in relation to other markers of the region can be achieved, and the role of m4 in AD inheritance might be evaluated.

2. Pedigree possibly linked to this region

In pedigree 0441 one allele of m4 segregates with AD. All 6 ill persons appear to share the markers of this region (m4, INS, HRAS) by descent. The highest lod score derived by making HRAS1/INS/m4 haplotypes was 1.1 at 0 recombination (penetrance = 50%). If the clinical investigators can succeed in extending this pedigree, it could be a potential candidate for linkage at the 11p15 region.

C. X-chromosome (Xq28)

Previous reports of linkage of colorblindness markers in the Xq28 region to bipolar disease prompted us to study the St-14 polymorphic DNA marker for Xq28

in our pedigrees of bipolar disease. Six pedigrees have been examined to date with no evidence of X-linkage. The cumulative LOD score is between -9.0 to -4.9 at theta values of 0 to 0.05 (assuming high penetrance, and model 2, see table 2, as in the published linkage reports).

Table 2: Xq28 Linkage Computation in 6 Pedigrees

High Penetrance	<u>0.0</u>	<u>.05</u>	<u>.10</u>	<u>.20</u>	<u>.30</u>
Model					
1	-10.76	-6.43	-4.69	-2.59	-1.30
2	- 9.00	-4.88	-3.36	-1.66	- .69
3	-8.26	-4.27	-2.86	-1.35	- .55

Model 1 = SA, BP; Model 2= Model 1 + UP; Model 3 = Model 2 + Eating disorders, cyclothymic.

Linkage heterogeneity was not detectable in this series. We conclude that linkage of manic-depressive illness to this region is not present in the families we have studied.

D. Other Candidate Genes (Dr. Berrettini)

Several genes, such as adrenergic or dopaminergic receptor genes, are hypothetically implicated as candidate genes in psychiatric disorders because of neuropharmacological evidence of changes in their density or activity during pharmacologic therapy of the disorders. The hypothesis that a particular gene is involved in an inherited pathophysiologic defect can be tested through linkage studies in a series of pedigrees with the inherited illness.

1. Beta₁-Adrenergic Receptor (Dr. Berrettini)

Using a 2.4 kb clone of the human beta₁-adrenergic receptor (B₁ AR) gene, 40 restriction enzymes were screened in a search for restriction fragment length polymorphisms (RFLPs). Bb1 I showed a biallelic RFLP, with bands at 6.2 and 4.7 kb. Gene frequency was .8 for the 6.2 allele and .2 for the 4.7 allele. Analysis of five bipolar pedigrees revealed that no one family showed a positive LOD score. Close linkage (0<.05) could be rejected assuming high penetrance for the illness gene. Additional pedigrees are being examined.

2. Beta₂-Adrenergic Receptor (Dr. Berrettini)

Using a 2.4 kb clone of the human beta₂-adrenergic receptor (B₂AR) gene, 45 restriction enzymes were screened in a search for RFLPs. Digestion of genomic DNA with Ban I revealed a biallel RFLP with bands at 3.7 and 3.4 kb. Gene frequencies were .25 and .75 respectively. Analysis of three bipolar pedigrees revealed that close linkage (0<.10) of the disease locus to the B₂AR gene could be excluded for these families assuming high penetrance for the disease locus. Additional families are being studied.

3. Alpha₂-Adrenergic Receptor (Drs. Hoehe and Berrettini)

Using a 5.5 kb clone of the human alpha₂-adrenergic receptor (AAR), 55 restriction enzymes were screened in a search for RFLPs. Dra I digestion of human genomic DNA revealed a biallelic RFLP (with bands at 6.0 and 5.5 kb) which is currently being evaluated in pedigrees of bipolar probands for possible linkage to the disease locus.

4. Phenylalanine Hydroxylase (PAH) (Dr. Gelernter)

The PAH gene is well studied and numerous PAH polymorphisms have already been identified. Previously described polymorphisms were used for the RFLP analysis; they are used clinically for prenatal diagnosis of phenylketonuria. These polymorphic sites occur in clusters at opposite ends of the gene. We found the most useful polymorphism to be the one generated by the enzyme EcoRV. Linkage in AD and SZ is being studied. Linkage to AD has been ruled out in 5 pedigrees.

E. Gene Mapping Methodology

1. DNA Amplification by Polymerase Chain Reaction (PCR) in Chromosome Walking and Jumping (Dr. Hsieh)

The polymerase chain reaction is performed according to the procedures described by manufacturer with modifications. We are developing methods to extensively map small regions of chromosome of interests such as the short arm of chromosome 11. We have employed the concepts of the construction of jumping library and the polymerase chain reaction for DNA amplification to explore the method of jumping or walking the chromosome in a specific fashion without screening a library. Several problems have been encountered such as the specificity of the annealing and amplification of the particular fragment and the ability to sequence the DNA fragment in an efficient way to obtain information for the consecutive jumping or walking.

2. Physical Mapping of Probes on 5q11-13

The strategy is to use transverse field gel electrophoresis to separate large DNA fragments ranging from 50 kb to 2000 kb. Gel inserts are prepared with lymphoblast cell lines derived from a selected member of one of the SZ pedigrees. Digestion with rare cutting restriction enzymes and subsequent Southern hybridization with GRL cDNA insert yield large size of hybridizing fragments. This blot will be rehybridized with other probes mapping to 5q11-13.

F. Development of Methods for Improved Resolution of Closely Spaced DNA Bands (Dr. Gejman)

The electrophoretic mobility of several DNA size markers of molecular lengths from 1.23×10^2 to 2.36×10^4 base pairs has been investigated in gels of 1% and 1.3% (w/v) by field inversion gel electrophoresis (FIGE) in vertical slabs. Pulsing times studied were in the range of 0.3/0.1 ms to 300/100 ms. (Pulsing times are given in milliseconds, in the format X/Y, where X is the forward

pulsing time and Y is the reverse time.) FIGE retards DNA migration; this effect is more marked for shorter pulsing times, and varies as a function of molecular length of the DNA fragment, down to fragments as small as about 0.5-1.0 kb, with the pulsing times we used. Several FIGE conditions were found which generate improved resolution patterns of DNA fragment size distribution. DNA separation improves by a more than a factor of 2 for fragments of 23.1-9.4 kb (300/100 ms) and for fragments of 4.4-2.0 kb (3/1 ms). FIGE has a less marked effect on DNA sized between 9.4 - 4.4 kb (60/20 ms). This procedure resulted in improved detection of closely spaced bands on Southern blots with H-ras oncogene. There are also implications for improved detection of restriction fragment length polymorphisms.

G. Molecular Cloning and Expression of Neurobiologically Important Genes

1. Molecular Cloning of the 87kDa substrate of Protein Kinase C (Dr. deMiguel in collaboration with Drs. Kligman and Patel)

Protein kinase C plays a key role in the phosphoinositol hydrolysis mediated pathway of signal transduction initiated by ligands of neurotransmitter receptors. The substrate proteins are not well defined. The present project attempts to determine the primary structure of the known 87kDa substrate (87-PKCS), study its expression, and isolate human cDNA and genomic clones.

Screening of a rat brain lambda gt11-cDNA library with an antiserum raised against the 87 kDa protein kinase C substrate (87PKCS) resulted in the isolation of 4 clones designated as PKCS - 7, PKCS 1, PKCS 19 and PKCS 18. Initial sequence data indicate that the antibody recognizes related proteins containing short sketches of homologous amino acid sequence. PKCS 7 turned out to be the GAP-43, a protein kinase C substrate which is abundantly expressed during neuronal growth and remodelling. PKCS-1 contains a 0.8 kb insert and a poly A tail. PKCS-19 has a 1 kb insert and also a poly A tail. Sequence analysis shows that PKCS-19 is distinct from PKCS-1 and that amino acid composition of PKCS-19 closely approximates that of the authentic 87 PKCS. The pattern of expression and size of transcript of these 2 clones are distinctly different in neuroblastoma cell lines and in lymphocytes. The absence of expression of PKCS-19 in a mutant lymphocyte previously shown to lack the 87-PKCS protein strongly suggest that this is the authentic clone. The isolation of the full-length cDNA clone is underway.

2. Isolation and characterization of calmodulin human genes (Dr. Detera and collaborators).

This work is done in collaboration with B. Sen Gupta, F. Friedberg, and W. O. McBride. Using a coding region probe of the human calmodulin cDNA isolated by our group, W. McBride has found calmodulin hybridizing sequences in 4 different chromosomes in somatic cell hybrids. Guided by this finding a chromosome specific library was screened with a coding region probe and a 1.1 kb clone was found. Sequence analysis reveal an intronless stretch of the gene showing extensive homology with the calmodulin cDNA which have been reported on previously. Amino acid substitutions are both radical and conservative, some occurring at the calcium binding domain. This clone has properties of a pseudogene.

III. Significance to Biomedical Research and the Program of the Institute and Proposed Course of Study

Use of molecular genetic probes as linkage markers in psychiatric disorders has great promise in resolving the inheritance of these disorders. Our current strategies are to study regions in which linkage or cytogenetic abnormalities are reported in these disorder, and to examine the role of candidate genes (on the basis of neurobiologic and neuropharmacologic data using linkage). Future plans include use of systematic gene mapping as well.

Other goals include improvement in genetic and physical mapping of chromosome regions of interest, such as 11p15 and 5q11-13, improved methods of RFLP resolution, and cloning of genes important in neurotransmission.

Publications:

Sen Gupta B, Friedberg F, Detera-Wadleigh SD. Molecular analysis of human rat calmodulin in complementary DNA clones. *J Biol Chem* 1987; 262:16663-16670.

Detera-Wadleigh SD, deMiguel C, Berrettini WH, deLisi LE, Goldin LR, Gershon ES. Neuropeptide gene polymorphisms in affective disorder and schizophrenia. *J Psychiat Res* 1987; 21:581-587.

deMiguel C, Bonner TI, Detera-Wadleigh SD. RFLP's associated with the substance P-neurokinin A gene (NKNA). *Nuc Acids Res* 1988; 16:1644.

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Hsieh W-T, Wells RD. Influence of negative supercoiling of the proximity of left-handed Z-DNA on the *Escherichia coli* lactose repressor-operator interaction. *J Biol Chem* 1987; 262:14576-14582.

Whitson PA, Hsieh W-T, Wells RD, Matthews KS. Influence of supercoiling and sequence context on operator DNA binding with Lac repressor. *J Biol Chem* 1987; 262:14592-14599.

Hsieh W-T, Whitson PA, Matthews KS, Wells RD. Influence of sequence and distance between two operators on interaction with Lac repressor. *J Biol Chem* 1987; 262:14583-14591.

Jaworski A, Hsieh W-T, Blaho JA, Larson JE, Wells RD. Left-handed DNA In Vivo. *Sci* 1987;238:773-777.

Wells RD, Amirhaeri S, Blaho JA, Collier DA, Hanvery J, Hsieh W-T, Jaworski A, Klysik J, Larson JE, McLean MJ, Wohlrab F, Zacharias W. Unusual DNA structures and the probes used for their detection. In: Wells, R. D., Harvey, S. C. eds. *Unusual DNA structures*. New York: Springer Verlag, 1987;1-21.

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Lentes K-U, Berrettini WH, Hoehe MR, Chung FZ, Gershon ES. A biallelic DNA polymorphism of the human β_2 -adrenergic receptor detected by Ban I. Nuc Acids Res, in press.

Berrettini WH, Hoehe MR. A biallelic polymorphism of the β_1 -adrenergic receptor gene detected with BglI. Nuc Acids Res, in press.

Gershon ES, Goldin LR. The outlook for linkage research in psychiatric disorders. J Psychiat Res 1987;21(4):541-550.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NG 00935-21 CNG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Plasmids and Small Genomes in Human Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C.R. Merrill	Chief, Biochemical Genetics	CNG, NIMH
Others:	L. Mitchell	Staff Fellow	CNG, NIMH
	D. Rath	Staff Biologist	CNG, NIMH
	B. Budowle	Chief, Forensic Science Research	FBI, Academy
	M.G. Harrington	Visiting Associate	CNG, NIMH
	T.Sunderland	Senior Staff	LCS, NIMH

COOPERATING UNITS (if any)

Forensic Science Research Group, FBI Academy, Quantico, Virginia

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Biochemical Genetics Section

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.25

PROFESSIONAL:

1.25

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To further the sections studies of mutational events in the mitochondrial genome, we have developed a rapid method of DNA sequencing: by employing the polymerase chain reaction and an amplification primer with a 5' Ligand to provide for a simplified method to prepare single stranded DNA, this DNA is easily sequenced by the Sanger technique. This procedure should greatly facilitate the study and diagnosis of human genetic diseases. Progress in adapting RNA:DNA duplexes for the rapid screening of genomic variations: mismatches, substitutions and deletions; has provided some preliminary evidence for heterogeneity in the human brain mitochondrial genome. Earlier experiments had suggested that mutations in the mitochondrial genome were very rare events. If the section's preliminary results are confirmed mutational events in the mitochondrial genome may provide some explanation for the pathophysiology associated with certain diseases and aging.

Studies of plasma DHEA Levels in patients with AIDS and Alzheimer's disease were stimulated by observations that high levels of DHEA may provide protection against viral illness. Plasma DHEA Levels were lowered in patients with these two diseases compared to age-matched controls. Investigations of DHEA Levels in AIDS and Alzheimers may provide information on whether the lowered levels found in these disease states are specific or merely an indication of disease in general. A double cross-over clinical trial has been initiated to determine whether the administration of DHEA has a beneficial effect in Alzheimer's disease patients.

PROJECT DESCRIPTION:

Mitochondrial DNA was initially discovered more than thirty years ago. Since then, the entire human mitochondrial genome has been sequenced and its length has been established at 16,569 bp. This unique DNA molecule has been shown to exist as a closed circular structure and studies have proven that it is inherited maternally. Additional research has also revealed that mitochondrial DNA is monoclonal in origin and has a high mutation rate (5 to 10 times that of the nuclear genome). This high mutation rate may be explained by the lack of both replicative and post-replicative DNA repair mechanisms in the mitochondria.

Due to these lack of repair mechanisms, the human mitochondrial genome might serve as a good indicator for the accumulation of somatic mutational events in postmitotic tissues. Studies of somatic mutational events may provide insights into pathophysiological processes. Germ line mitochondrial mutational events have already provided useful in anthropological studies and they may further our ability in establishing forensic individuality.

The section has developed methods to examine the intra-individual somatic heterogeneity of the mitochondrial genome in the central nervous system. The brain is a highly aerobic tissue which relies heavily on its mitochondrial population for critical metabolic processes. Many of these respiratory processes result in the generation of free radicals, which have the potential to cause mutational events in interactions with DNA. The close proximity of the mitochondrial DNA to the site of production of free radicals coupled with the apparent lack of DNA repair mechanisms in the organelle suggests that somatic mutations could accumulate in the mitochondrial genome of postmitotic tissues with aging and in certain disease processes.

Studies of intra-individual somatic cellular mitochondrial heterogeneity have been initiated by purifying mtDNA from human brain tissue samples obtained at autopsy. A 548 bp fragment which represents 80% of the cytochrome oxidase subunit II has been cloned into a pUC vector and more than 20 clones have been isolated which contain the desired insert. The fragment has also been subcloned into a pSP vector which contains an SP6 promotor to allow the synthesis of RNA probes homologous to the cloned insert. RNA probes have been synthesized using ³²P UTP. These probes are then hybridized to DNA from the pUC clones. If any base substitutions are present, they are detected by cleavage with ribonuclease-A at the mismatched regions in the RNA:DNA duplexes followed by denaturing gel electrophoresis. This method detects greater than 70% of the mismatches present and can be used more efficiently to examine the mitochondrial genome in an individual for the accumulation of mutational events than conventional sequencing technologies. In this manner, the variations which may have accumulated through physiological or pathophysiological events over the course of a lifetime as a result of random mutations in a specific regions of an individual's brain can be evaluated.

Preliminary results seem to indicate that variation does exist in a single individual's mitochondrial population.

The effect of maternal transmission on age at onset of Huntington's disease is being examined by restriction enzyme analysis and the RNA:DNA duplex methodology. Brain samples from individuals with known maternal or paternal transmission have been obtained. Whole cell DNA has been extracted from these tissues and they have been digested with a total of six restriction enzymes. The Southern blots from these samples are being hybridized to nick-translated probes which represent the entire mitochondrial genome. In this manner, deletions, additions or polymorphisms which may play a role in the age of onset of the disease will be detected. Additionally, these samples will be examined using the RNA:DNA duplexes methodology.

The section has also continued to develop procedures to permit rapid direct sequencing of DNA's, including human MtdNA. These sequencing techniques permit a detailed examination of any mutational events discovered in the screening program described above. Direct sequencing with elimination of the need for cloning should save the time currently needed to determine a DNA sequence.

With the recent availability of Taq DNA polymerase, we have been investigating strategies for direct sequencing by employing the Polymerase Chain Reaction (PCR). Our attempts to replicate MtdNA sequencing followed the protocol published by Wrischnick et al (Nuc Acids Res 15, 529-542, 1987). However, our initial attempts were not very successful due to difficulties in obtaining single stranded DNA for the Sanger DNA sequencing reactions. The Wrischnick method relies on the use of DNA strand denaturing agarose gels to obtain the single stranded DNA needed to sequence. However, these gels require a high degree of strand bias to achieve separation of the single strands. Many regions of human DNA do not have the level of bias necessary for separation using this technique.

We have tested several strategies to obtain single stranded DNA following the PCR, including: Denaturing agarose gel electrophoresis, Double amplification (where the PCR product DNA is gel purified, extracted, and reamplified in the presence of only one primer), PCR with limited availability of one primer (so that the limited primer is exhausted during amplification and only the strand made with the unlimited primer is produced), and the use of a complementary strand blocking primer during sequencing (for use when the template is present in double stranded form, the blocker is complementary to the region of the non-template strand immediately 3' to the sequencing primer: this is to prevent the non-template strand from re-annealing). None of these techniques were very successful, so we have totally eliminated the non-template strand by linking a ligand to the 5' end of one amplification primer used in the PCR. After amplification, the non-template strands are removed by affinity chromatography. By this technique, we have generated DNA which is easily sequenced by the Sanger technique. Our method of affinity generation of single stranded

DNA is not dependent on the composition of the DNA to be sequenced, and should have wide applicability for DNA sequencing.

Some small genomes found in human cells may be of exogenous origin, such as the AIDS viral genome. There is considerable evidence from mammalian experiments that some endogenous substances may offer some protection from the pathological effects of certain exogenous viral genomes. One of these compounds is dehydroepiandrosterone. In rodents, a diet supplemented with dehydroepiandrosterone protects against breast tumors, diabetes, obesity, auto-immune nephritis, viral infections and chemically-induced tumors of the colon and lung.

Death from all causes has been correlated with decreased levels of plasma dehydroepiandrosterone. The large number of patients with acquired immune deficiency syndrome, and its poor prognosis, prompted us to investigate whether there is a correlation between infection with human immunodeficiency virus and plasma dehydroepiandrosterone levels.

Three additional avenues of investigation have suggested to us that dehydroepiandrosterone may be influenced by and/or may influence infections with human immunodeficiency virus. Firstly, plasma levels of dehydroepiandrosterone are age-dependent, with low levels in childhood and in older adults, the ages which have the poorest prognosis for patients with acquired immune deficiency syndrome. Secondly, there are many disease correlations with plasma dehydroepiandrosterone: in humans, low plasma dehydroepiandrosterone levels have been recorded in women with breast tumors;

Thirdly, in human cell cultures, dehydroepiandrosterone protects against chemical mutagenesis and viral transformation. The therapeutic benefit that occurs in such diverse rodent disease states may arise from metabolism of dehydroepiandrosterone to an array of biologically active steroids, both of androgenic and estrogenic type.

To investigate the possible role of dehydroepiandrosterone in the body's reaction to infection with the human immunodeficiency virus, we measured plasma total dehydroepiandrosterone levels in an age-matched blind study: dehydroepiandrosterone levels were lower in eight apparently well persons with positive human immunodeficiency virus serology compared to nine serologically negative controls (2145ug/ml +/- 621: 2964ug/ml +/- 866; $p < 0.05$). Ten individuals with acquired immune deficiency syndrome had still lower values (1443ug /ml +/- 834; $p < 0.001$). Plasma total dehydroepiandrosterone levels were measured by radioimmunoassay. The intra- and interassay coefficients of variation were 4.1% and 12.2%, respectively.

The observations of diminished plasma dehydroepiandrosterone levels in patients infected with the human immunodeficiency virus, and the reports of the protective effects of dehydroepiandrosterone in the animal and human cell culture studies suggest that further

investigation of the role of dehydroepiandrosterone in human immunodeficiency virus infections may be merited.

We also measured dehydroepiandrosterone levels in patients with Alzheimer's disease and found levels in these patients significantly lowered in comparison to age-matched controls.

SIGNIFICANCE TO BIOMEDICAL RESEARCH:

A method that permits sequencing unpurified DNA from lysed cells in a matter of hours has been developed by coupling the DNA polymerase chain reaction, with a rapid method for separating one of the amplified strands. The sequencing reaction products can be detected by silver staining. These methods should facilitate the laboratory's studies involving inherited diseases and our collaborative efforts with the FBI on the development of techniques for individualization. These methods should also facilitate the laboratory's studies on mutagenesis in the brain.

The Section's progress in the utilization of RNA:DNA duplexes for the rapid screening of genomic variations, including mismatches, substitutions and deletions has provided some preliminary evidence for heterogeneity in the human mitochondrial genome. Previous experiments suggested that mutations in the mitochondrial genome were very rare events. If the Section's preliminary results are confirmed, they may provide some explanation for some of the pathophysiology associated with disease and perhaps aging.

Preliminary results indicate that AIDS patients have significantly lower levels of dehydroepiandrosterone than non-symptomatic HIV positive individuals while normal age matched controls have the highest levels. In view of experiments with rodents which indicate that high levels of DHEA provide protection against some viral diseases these results may indicate that the administration of DHEA may be of some value in the treatment, of AIDS. The use of DHEA should also be examined in Alzheimers disease given the lowered levels detected in age matched patients.

The Section's development of a highly sensitive hybridization which permits the mapping of exogenous DNA genomes in tissue slices should provide evidence of the distribution of viruses, such as HIV, in infections of the brain.

PROPOSED COURSE OF RESEARCH:

The section proposes to continue to develop the rapid sequencing and hybridization methods to facilitate studies of genetic diseases. These methods should also prove useful in our collaboration with the Federal Bureau of Investigation's Forensic Science Research Group. We plan to test the feasibility of using mitochondrial and nuclear DNA's isolated from forensic samples for identification purposes with this rapid sequencing technique. Studies of mitochondrial genomes in normal and disease states are

now in progress.

Examination of large numbers of independent clones from specific regions of the mitochondrial genome from postmitotic tissue, such as the brain, should provide evidence for or against the accumulation of mutational events in this genome with aging, or during disease processes.

Further investigations of DHEA levels in AIDS and other diseases affecting the CNS should provide information on whether the lowered levels found in Alzheimer's disease and AIDS are specific or merely an indication of disease in general. A double cross-over clinical trial has been designed with Dr. Sunderland of the LCS, NIMH, to determine whether the administration of additional DHEA has a beneficial effect in Alzheimer's disease patients.

PUBLICATIONS: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00941-08 CNG
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical Genetics and Metabolic Diseases.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 30%;"> PI: C.R.Merrill Others: M.G.Harrington S.Charya D.F.Hochstrasser A.C.Hochstrasser </div> <div style="width: 40%;"> Chief, Biochemical Genetics Visiting Associate Staff Associate Guest Researcher Guest Researcher </div> <div style="width: 25%;"> CNG, NIMH CNG, NIMH CNG, NIMH CNG, NIMH CNG, NIMH </div> </div>		
COOPERATING UNITS (if any) <div style="text-align: center; margin-top: 10px;"> NIMH, NINCDS, Harvard Medical School, California Institute of Technology, University of Virginia, Charlottesville. </div>		
LAB/BRANCH Clinical Neurogenetics Branch		
SECTION Biochemical Genetics Section		
INSTITUTE AND LOCATION NIMH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 4.5	PROFESSIONAL: 3.5	OTHER: 1
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="margin-top: 10px;"> <p>Methods to investigate <u>complex protein mixtures</u> have been improved: Electrophoresis and staining of proteins from <u>cerebrospinal fluid</u> (CSF) and brain tissue have increased detection from 300 to 1500 and 1000 to 3500 respectively.</p> <p>Disease-associated proteins in the CSF have been further investigated. Purification and <u>partial amino acid</u> sequences have been obtained from 6 proteins: One of the proteins is identified as a <u>transthyretin molecule</u>, twice the molecular weight of the usual protein, and absent from the plasma. The other 5 proteins are not homologous to any of the known sequences in the NBRF protein database. Of these 5, two are diagnostically important in <u>Creutzfeldt-Jakob disease</u>, one is reduced in schizophrenia, and two are increased in multiple sclerosis. <u>Synthetic peptides</u> have been made to these peptides, and antibodies are being produced, in order to develop sensitive rapid and accurate immunoassays for these newly characterized proteins.</p> <p>Two-dimensional electrophoretic survey studies have been continued with various disease model systems. Brains from inbred mice strains have been studied, with a normal protein reference being established for common strains, in order to study neurological mutants that have been bred on a similar genetic background. Hamster brain proteins have been studied, in normal, heterozygotic autosomal dominant <u>circadian rhythm</u> mutants and <u>homozygotic mutants</u>. Multiple protein alterations have been identified, and further investigations are in progress to define which of these proteins is associated with variations in circadian rhythm.</p> <p>Further developments to advance the resolution and detection of proteins has resulted in the synthesis of a new crosslinker which allows the polymerization of gels with better physical strength, less hydrophilicity, and better protein separation by two dimensional gel electrophoresis. Its probable lower affinity for silver ions and its greater resistance to hydrolysis also delays the appearance of a background stain with <u>ammoniacal silver nitrate</u>.</p> </div>		

COLLABORATORS:

T.Sunderland	Staff Fellow	LCS NIMH
D.Asher	Senior Staff	CNSS NINCDS
D.C.Gajdusek	Chief, Lab.of Central Nerv.Sys.Studies	CNSS NINCDS
P.Brown	Neurologist	NINCDS
E.F.Torrey	Staff Psychiatrist	St. Elizabeths Hosp. Wash., D.C.
L.Hood	Chairman, Biology Dept.	California Inst. of Tech.
S.Kent	Scientist	California Inst. of Tech.
R.Aebersold	Scientist	California Inst. of Tech.
R. Sidman	Prof.Neuropath	Dept.Path.Harvard Med.School
P.Neumann	Staff Fellow	Dept.Path.Harvard Med.School
R.O'Neill	Staff Fellow	DMN NINCDS
B.Martin	Visiting Associate	CNB NIMH
M.Miller	Senior Staff	NCI
D.Hunt	Professor	Dept.Chem. Univ. of Va.
M.Menaker	Chairman	Dept.Biol. Univ. of Va.

PROJECT DESCRIPTION:

Introduction of two dimensional high-resolution electrophoresis (2DE) by O'Farrell in 1975, permitted an order of magnitude increase in the resolution of proteins. This advance in separation technology, when coupled with the high sensitivity of silver stains developed by this section have permitted the visualization and determination of the charge and mass of hundreds, and in some cases thousands, of proteins from body fluids and tissues.

Proteins that are separated by 2DE are mapped by their charge and mass. However, they must also be further characterized to study their origin and function. This can best be achieved by: confirmation of identity by co-migration with a purified protein on a 2DE-gel, recognition by a specific antibody on a western-blot of a 2DE gel, or by obtaining a partial amino acid sequence of the protein. The origin and function of the protein may then be directly obtained by referencing the protein and nucleic acid sequence data banks. These data banks may also give access to the role of the specific protein in the metabolic network. Alternatively, if the protein contains a novel sequence, then such a sequence provides an initial focus for further studies.

Two major strategies, utilizing these technologies, have been employed to search for diagnostic markers and clues concerning the underlying pathophysiology of schizophrenia and other diseases affecting the CNS. One strategy is to search for disease-associated proteins and protein changes in tissues and body fluids (the disease-association strategy). The other strategy utilizes genetically variant proteins, polymorphic proteins, to establish genetic linkage with a disease locus in families and sib-pairs (the polymorphism strategy).

Surveys for human disease-associated brain protein changes are hampered by a lack of knowledge of normal brain proteins and their variations during normal physiological and pharmacological events.

In this regard, studies of CNS proteins in non-human mammalian models are likely to provide control data that cannot be obtained in human studies. Although 2DE has been employed to study protein alterations in specific regions of the brain following experimental manipulations there are few studies of proteins in regions of the normal mammalian brain. This section collaborated on a limited quantitative analysis of the male rat brain. Of 91 proteins examined from 25 different brain regions the majority of proteins varied less than 4-fold in concentration between the neuroanatomical sites. However, 10% of the proteins varied widely, with concentrations ranging more than 10-fold between the regions with the highest and lowest amounts. The number of proteins examined in their study was limited by the lack of fully automated gel matching and quantitation facilities. Furthermore, in the initial study circadian rhythm effects were not controlled for. In ongoing studies with M. Mennicker, at the University of Virginia, we have found a number of proteins that have significant quantitative day/night variations. By the examination of an animal model with mutations in the circadian rhythm we hope to begin studies on the molecular basis of a biologic "clock".

Examination of cerebrospinal fluid (CSF) proteins offers the possibility of detecting abnormal gene products associated with diseases of the central nervous system. Although most of the proteins in spinal fluid are believed to be filtration products of the blood, some of the spinal fluid proteins are derived from brain and spinal cord. Of these there is a subclass that may represent neurotrophic factors which may be altered in disease and physiologic states. Acetylcholinesterase activity in the CSF has been shown to increase after treatment with chlorpromazine while the enzyme dopamine-beta-hydroxylase has been found to vary with central noradrenergic activity.

High-resolution 2DE and silver staining has permitted the visualization of more than 300 spinal fluid proteins of which 26 have been identified by this section, some of which appear to be CSF-specific.

In extensive studies of CSF from normal controls and individuals with various diseases of the nervous system, a 25KD protein has been found which appears to be associated with Parkinson's disease. In addition, four other disease-associated proteins have been found in: Creutzfeldt-Jakob disease, herpes simplex encephalitis, the Guillain-Barre syndrome, multiple sclerosis, Parkinson's disease and schizophrenia. Two of these CSF proteins (with molecular weights of 29KD and 26KD, and iso-electric points of 5.1 and 5.2, respectively) were found in 100% of the patients with Creutzfeldt-Jakob disease, and 50% of the patients with herpes simplex encephalitis. The other two proteins (both have molecular weights of 40KD and iso-electric points of 5.7 and 5.9), have been observed in: Creutzfeldt-Jakob disease (67% of the patients), herpes simplex encephalitis (90%), the Guillain-Barre syndrome (100%), multiple sclerosis (13%), Parkinson's disease (12%) and schizophrenia (32%).

The new proteins observed in the CSF of the schizophrenic patients are believed to have originated within the CNS, as they could not be detected in 10-fold concentrates of CSF from normal volunteers, or in the patients' serum. It is unlikely that the appearance of these proteins is drug-related, as there was no common drug utilized in the treatment of the patients that displayed the abnormal proteins compared to those patients that did not have abnormal CSF proteins. Furthermore, it may be of some importance that all of the non-schizophrenic patients that displayed the abnormal 40KD CSF proteins have diseases of suspected viral origin. The common etiology of these disease-associated proteins is not known, however, the 32% of schizophrenic patients displaying these abnormal CSF proteins may belong to a subclass with a viral component in their disease process.

Purification and partial amino acid sequences have been obtained from 6 CSF proteins: one of the proteins is identified as a transthyretin molecule, twice the molecular weight of the usual protein, and absent from the plasma. The other 5 proteins are not homologous to any of the known sequences in the NBRF protein database. Of these 5, two are diagnostically altered in Creutzfeldt-Jakob disease, one is reduced in schizophrenia, and two are increased in multiple sclerosis. Synthetic peptides have been made to these peptides, and antibodies are being produced, in order to develop a fast accurate immunoassay for these newly characterized proteins.

In an effort to visualize more of the "trace" proteins which are often affected in disease states the Section continued its efforts to advance the arts of protein separation and detection.

In all detection methods, sensitivity depends on signal to noise ratio. To increase this ratio, one has to either increase the signal, or decrease the noise. When silver staining was first introduced as a general detection method for proteins separated on polyacrylamide gels, one of the limitations in the sensitivity of the method was caused by a background staining. Photographic "reducers" containing sodium thiosulfate have been employed to reduce this background staining. However, thiosulfate affected not only the background, but also the silver densities in the stained protein bands or spots. Recent chemical studies have permitted a better understanding of some of the mechanisms underlying this background staining. These studies have led to methods that delay and reduce the appearance of the background staining and improve resolution of proteins on 2-dimensional gel electrophoresis.

Basic and sulfur containing amino acids have been shown by this Section to be essential in the detection of peptides by the silver staining reaction. Furthermore, a single sulfur or basic amino acid is usually not sufficient; there appears to be a requirement for cooperative effects between two or more of these groups. This knowledge led us to a hypothesis that the amide groups of bisacrylamide in the gel might be partially responsible for background staining. A number of studies were performed to test this hypothesis.

SUBJECTS:

All the human tissue samples were obtained from patients that were diagnosed by our clinical collaborators, using nationally recognized diagnostic criteria. All animal samples were obtained from our collaborators in Harvard Medical School and the University of Virginia.

LABORATORY PROCEDURES:

Protein microsequencing (about 50 picomoles or less) has been performed in collaboration with three groups: continued work with L.Hood's lab at CalTech; local work with B.Martin at NIMH; both of these collaborations involve gas/liquid phase Edman chemistry. Thirdly, work with D.Hunt's lab in Virginia has been developed for obtaining multiple segments of sequence from tryptic digests of proteins by Fourier transformation of data from triple quadrupole, fast atom bombardment mass spectrometry.

Computer-assisted analysis of complex two-dimensional gels continues to be an area for development. Collaborative work with the NCI group (M.Miller) and the computer science department at the University of Geneva (D.Hochstrasser) is providing the Section with a number of options in these developments.

The laboratory continues to employ numerous protein purification procedures, including chromatography, and electrophoresis methodologies. The electrophoretic methods include both one and two dimensional electrophoresis. Proteins are detected by silver, dye and immunological staining.

MAJOR FINDINGS:

1. CSF protein studies:

Studies of two-dimensional gels (2DE) in recent years with our colleagues has led to the identification of over 30 disease-associated protein changes in the CSF of patients with various diseases of the nervous system. The identity of the majority of these proteins is not known. Partial amino acid sequences have been obtained for 6 proteins of these proteins: Proteins 130 and 131 (Mr 30000; pI 5.1, 5.2), known to be of diagnostic value in Creutzfeldt-Jakob disease, have each had 10 amino acids sequenced. Synthetic peptides have been produced, and antibodies are being raised; Protein 5 (Mr 28000; pI 5.8), quantitatively reduced in patients with schizophrenia, has been digested with trypsin, and 5 fragments have been sequenced between 8-19 amino acids long. Synthetic peptides have been made for antibody production, and synthetic oligonucleotides for the corresponding cDNA have also been synthesized; work on selecting and sequencing the gene for protein 5 is in progress. Proteins na1 and na2 (Mr 35-39000; pI 4.9-5.3) from plasma, also known to be

increased in CSF in patients with multiple sclerosis, have had 11 and 13 amino acids sequenced from the amino terminus. Synthetic peptides for these peptides have been made for antibody production.

The 5 proteins described above have all been compared to the protein sequence database at the NBRF, and there are no identical matches. The sixth protein, protein 16, has the 15 N-terminal amino acids of transthyretin, which is a protein of half the molecular weight. Protein 16 (Mr 35,000; pI 5.6) is of interest as it is not present in the plasma of normal persons.

2. Studies on genetic models of disease:

The studies utilize the genetic and experimental control that is impossible to achieve in human studies. Once protein changes in the animal model are established, then they should be directly of relevance to the study of human disease:

a) In collaboration with Sidman and Neumann (Harvard Medical School), different methods of examining the 2DE protein patterns on micropunched brain regions of mice have been assessed, and now over 3,500 protein spots can be detected. Preliminary studies in inbred strains are underway: over 30 differences have been identified between C57BL6 and DBA6J mice. This baseline is critical for studies on neurological mutants, work that is just starting.

b) In collaboration with Menaker (U. of Virginia), we have initiated experiments with a hamster which has an autosomal dominant mutation which affects it's circadian rhythm. Protein alterations in the mutant compared to the wild type have been identified, and further work in assessing these protein changes is in progress.

3. Detection of proteins:

Our studies to improve protein detection and resolution has resulted in the development of new acrylamide crosslinking agents. We obtained evidence that the amide groups of methylene-bisacrylamide play a role in the background staining with the ammoniacal silver stains. Contaminants in methylene-bisacrylamide preparation, or formaldehyde formation by the degradation of methylene-bisacrylamide, may play an even greater role in background formation. The synthesis of a new crosslinker, diacrylyl-piperazine, and the design of a new "catalyst" system results in polyacrylamide gels with better separation of proteins and significantly delayed appearance of the background with the ammoniacal silver stain. The increased signal to noise ratio; obtained with these developments has improved the definition of spots on silver stained two dimensional gels, and their detection by computer.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE:

The ability to sequence abnormal and normal proteins that have been visualized by high resolution two dimensional electrophoresis

offers the institute the possibility of utilizing these technique for the development of diagnostic tests and probes to further our understanding of CNS diseases. Protein sequence information, even partial sequence information, allows the production of synthetic peptides and oligonucleotide probes. The synthetic peptides can be used to raise antibodies which permit the development of rapid highly sensitive immunological assays. Such assays should facilitate testing for the presence of the abnormal proteins in patient populations. This research should provide a number of new highly specific diagnostic tests for diseases of organic origin.

Oligonucleotide probes will facilitate the isolation of the gene for the abnormal or disease related proteins. One of the major questions in some diseases, such as schizophrenia, concerns their possible viral origin. If an oligonucleotide probe to a disease related protein does not map to the endogenous human genome it may be used to search for exogenous, possibly viral genomes.

Studies concerning the detection and resolution of proteins has resulted in a fundamental advance in the development of new cross-linking agents. These cross-linking agents provide for gels with higher protein resolution, due to their decreased interaction with the protein and for a decreased background with silver staining. This latter advance provides for the detection of a greater number of trace proteins which are often important in disease states. The section has also developed new electrophoretic protocols and methods which have increased the number of proteins that can be visualized in a single gel. We used to be able to visualize about 300 proteins from 80 microliters of spinal fluid; with the new cross-linkers and protocols we have been able to visualize 1300 proteins from 25 microliters of CSF. This advance should permit the discovery of many new disease related proteins.

PROPOSED COURSE OF THE PROJECT:

The Section plans to increase its ability to sequence abnormal and normal proteins. In this regard, we have initiated a collaborative effort to explore the use of the tandem mass Spectrophotometer to augment the N-terminal chemical methods that we are pursuing with Drs. Hood, Aebersold and Martin. We hope that the tandem mass Spectrophotometer will be able to provide increased sensitivity and information concerning peptides with blocked N-terminal groups. These sequences will be utilized for the production of synthetic peptides and oligonucleotide probes. The synthetic peptides will be used to raise antibodies to permit the development of rapid highly sensitive immunological assays. Such assays should facilitate testing for the presence of the abnormal proteins in patient populations. This research should provide a number of new highly specific diagnostic tests for diseases of organic origin. We also plan to use the oligonucleotide probes to isolate genes associated with abnormal or disease related proteins. One of the major questions in some diseases, such as schizophrenia, concerns their possible viral origin. If an oligonucleotide probe to a disease related protein does not map to the endogenous human genome

it may be used to search for exogenous, possibly viral genomes.

The section also proposes to continue studies concerning the detection and resolution of proteins. The advances we have made offer the possibility of a rapid general protein assay in the nanogram range. It should be able to develop this assay within the next six months.

One of the major difficulties in extending these techniques involves the need for automated methods for the analysis of the complex two dimensional electrophoretograms. The section has established collaborative agreements with Drs. Miller and Lempkin in the NCI and Dr. Hochstrasser at the University of Geneva. With the help of these collaborations we hope to establish reliable methods for the automation of quantitative analysis of the large number of gels which are needed for clinical studies.

PUBLICATIONS:

Steven A.C., Bisher M.E., Harrington M.G. and Merrill C.R.: All that glitters is not gold: The physical basis of protein coloration in silver-stained gels. In Bailey G.W. (Ed.): Proceedings of the 45th Annual Meeting of the Electron Microscopy Society of America. San Francisco Press, San Francisco, Cal.: pp. 940-941, 1987.

Gershon E.S., Merrill C.R., Goldin L.R., Delisi L.E., Berrettini W.H. and Nurnberger J.I. Jr.: The role of molecular genetics in psychiatry. Biol.Psych. 22: pp. 1388-1405, 1987.

Merrill C.R.: Detection of proteins separated by electrophoresis. Advances in Electrophoresis 1: pp. 111-139, 1987.

Merrill C.R., Bisher M.E., Harrington M.G. and Steven A.C.: Observations concerning the colors of silver-stained protein bands in polyacrylamide gels and the development of silver grains of different sizes. Proc.Natl.Acad.Sci. U.S.A. 85: pp. 453-457, 1988.

Merrill C.R. and Harrington M.G.: Two-dimensional protein maps in studies of schizophrenia. Schizophrenia Bulletin, National Institute of Mental Health. In Press.

Harrington M.G. and Merrill C.R.: Cerebrospinal fluid protein analysis in diseases of the nervous system. J.Chromatography. In Press.

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Hochstrasser D.F., Harrington M.G., Hochstrasser A.C. and Merrill C.R.: Methods for increasing the resolution of two-dimensional protein electrophoresis. Analytical Biochemistry. In Press.

Hochstrasser D.F., Harrington M.G., Hochstrasser A.C. and Merrill C.R.: Improved Separation and Detection of Proteins with Reduced Background Silver Staining. Electrophoresis 88. In Press.

Hochstrasser D.F., and Merrill C.R.: "Catalysts" for Polyacrylamide Gels Polymerization Which Improve the Detection of Proteins by silver staining. Applied and Theoretical Electrophoresis. In Press.

Croxson M., Brown P., Synek B., Harrington M.G., Firth R., Clover G., Wilson J. and Gajdusek D.C.: A new case of Creutzfeld-Jakob Disease associated with human growth hormone therapy in New Zealand. Neurology. In Press.

Marzewski D., Tofigi R., Harrington M.G., Merrill C.R. and Brown P.: Creutzfeld-Jakob disease following pituitary-derived human growth hormone: A new American case. Neurology. In Press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01836-10 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

GABA/Receptors in the Central Nervous System: Biochemistry to Behavior

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S.M. Paul, Chief, NS, NIMH

Others: J. Crawley	Sr Staff Fellow	NS, NIMH
S. Cottingham	PRAT Fellow/Guest Researcher	NS, NIMH
P. Montpied	Visiting Fellow	NS, NIMH
A. Lingford-Hughes	Visiting Fellow	NS, NIMH
P. Suzdak	PRAT Fellow	NIGMS, NIMH
A. Morrow	PRAT Fellow	NIGMS, NIMH

COOPERATING UNITS (if any)

Laboratory of Bioorganic Chemistry, NIADDK; Unit on Molecular Neurogenetics, NS, NIMH; Pharmacology Research Associate Training Program, NIGMS; LSU School of Medicine, New Orleans, LA

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

3.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Benzodiazepines interact with a specific neuronal membrane receptor to initiate a series of neuronal events resulting in an enhancement of GABA-mediated chloride permeability. The latter results behaviorally in the major pharmacological actions of benzodiazepines, namely their anxiolytic, anticonvulsant, hypnotic and muscle relaxant actions. In addition to benzodiazepines, a variety of sedative/hypnotic agents of the minor tranquilizer class (e.g. the barbiturates) appear to interact with one or more components of the benzodiazepine/GABA receptor complex, and thus the latter has been proposed as a common site of minor tranquilizer action. Several aspects of the benzodiazepine/GABA receptor complex are currently being studied. Recent work has employed an in vitro system for measuring GABA receptor-effector coupling in a subcellular preparation from rat brain (the synaptoneurosome). This technique has greatly facilitated studies on the regulation of the GABA receptor-coupled chloride ion channel. Using this method, we have studied the interaction of ethanol with the GABA receptor complex and have found that ethanol, related short-chain alcohols and several anesthetic agents are capable of stimulating this receptor and at pharmacologically-relevant concentrations. In related studies we have identified a novel imidazobenzodiazepine, Ro15-4513, which blocks both the in vitro effects of ethanol on GABA receptor-mediated $^{36}\text{Cl}^-$ uptake as well as many of the behavioral effects of ethanol. Chronic administration of ethanol to rats results in a decrease in GABA receptor-mediated $^{36}\text{Cl}^-$ uptake in synaptoneurosomes, an effect that is reversible since it is not observed after the ethanol withdrawal syndrome. In other studies we have examined the use of the radiolabelled benzodiazepine receptor antagonist Ro15-1788 for measuring benzodiazepine receptors in vivo. Our results have validated the suitability of this technique and have demonstrated significant effects of barbiturates, naturally-occurring steroid hormones, ethanol and "stress" on benzodiazepine receptors in vivo.

PROFESSIONAL PERSONNEL:

A. Weizman	Guest Researcher	NS, NIMH
R. Weizman	Visiting Scientist	NS, NIMH
F. Vocci	Guest Researcher	NS, NIMH
B. Martin	Visiting Scientist	NS, NIMH
E. Ginns	Neurologist/Biochemist	NS, NIMH
P. Skolnick	Pharmacologist	LBC, NIADDK
L. Miller		LSU

PROJECT DESCRIPTIONObjectives:

1. To characterize the interaction of anxiogenic and anxiolytic compounds with the benzodiazepine/GABA receptor complex at the molecular/cellular, neurophysiologic and behavioral levels.
2. To understand the mechanism(s) responsible for the stress-induced "sensitization" and drug-induced desensitization of this receptor complex (including the possible involvement of steroid hormones); and to unravel whether changes in the ionophore underlie the development of tolerance to sedative/hypnotic/anxiolytic drugs.
3. To use the GABA receptor-coupled Cl^- ion channel complex as a model of other neurotransmitter-gated ion channels.
4. To explore basic neurochemical mechanisms of anxiety, fear and stress as they relate to the many clinical and medical problems associated with stress.

Methods Employed:

(See: 1986 Annual Report, Project Number Z01 MH 01836-08 NS, GABA/Receptors in the Central Nervous System: Biochemistry of Behavior)

Major Findings:

Agents that perturb one or more of the components of the benzodiazepine-GABA receptor chloride ionophore complex (e.g., benzodiazepines, GABA and GABA mimetics like muscimol and barbiturates) have been shown to increase chloride conductance in both electrophysiological preparations and intact cells. Attempts to develop a quantitative measurement of chloride flux in a "cell-free" preparation (in order to explore the functional relationships between the binding sites associated with the receptor complex) have generally been unsuccessful. We have developed a method for measuring $^{36}\text{Cl}^-$ flux in "filtered synaptoneuroosomes." The filtered synaptoneurosome preparation was employed because it has been shown to contain both presynaptic nerve endings and attached postsynaptic densities. We have previously shown that barbiturates, including pentobarbital, cause a concentration dependent increase in the efflux and uptake of $^{36}\text{Cl}^-$ efflux from synaptoneuroosomes which is reversed by the chloride ionophore antagonist, picrotoxin. A good correlation ($r = 0.90$, $p < 0.01$) was observed between the

potencies of a series of barbiturates in increasing $^{36}\text{Cl}^-$ efflux from preloaded synaptoneurosomes and their anesthetic potencies in mice. Barbiturates also potentiate the stimulation of $^{36}\text{Cl}^-$ uptake induced by GABA agonists such as muscimol. In addition to barbiturates, a number of naturally-occurring substances including the A ring reduced metabolites of progesterone and deoxycorticosterone (3 alpha 5 alpha dihydroprogesterone and tetrahydrodeoxycorticosterone) have been found to be potent barbiturate-like modulators of the GABA receptor-gated chloride ion channel. The latter have also been shown to have anxiolytic and hypnotic effects in rats and mice.

Work over the past several years has documented that both benzodiazepines and barbiturates stimulate $^{36}\text{Cl}^-$ efflux or uptake via a pharmacologically-relevant GABA_A receptor. Since the latter drugs show cross-tolerance and cross-dependence with alcohol we have further examined the effects of ethanol (the most commonly used anxiolytic/hypnotic/intoxicant) on the GABA receptor coupled Cl^- ion channel. Our data demonstrate that ethanol at concentrations from 20 to 100 mM stimulate $^{36}\text{Cl}^-$ uptake via the GABA_A receptor, since this effect is blocked by the GABA_A receptor antagonists bicuculline and picrotoxin and not by a variety of other neurotransmitter receptor antagonists. In addition to ethanol, many short-chain alcohol and anesthetic agents tested stimulate $^{36}\text{Cl}^-$ uptake. These data suggest that ethanol (and related alcohols) produce at least some of their behavioral effects via an interaction with the GABA receptor-coupled Cl^- ion channel and most likely by altering the membrane (lipid/protein) microenvironment of the receptor, rather than directly binding to the receptor protein itself. In related experiments we have found that the imidazobenzodiazepine Ro15-4513, which is a derivative of the benzodiazepine antagonist Ro15-1788, blocks the ability of ethanol to stimulate $^{36}\text{Cl}^-$ uptake in vitro. Moreover, Ro15-4513 fails to block either muscimol- or pentobarbital-stimulated $^{36}\text{Cl}^-$ uptake at concentrations ≤ 1 μM . In behavioral studies, Ro15-4513 also blocks the effects of low (1 g/kg) as well as moderate (2 g/kg) doses of ethanol in rodents; but not higher (≥ 4 g/kg) doses of ethanol. The effects of Ro15-4513 in blocking both ethanol-stimulated $^{36}\text{Cl}^-$ uptake in vitro and ethanol-induced intoxication in vivo were not mimicked by the full inverse agonist BCCE or the partial inverse agonist FG-7142. The latter finding suggests that Ro15-4513 has "selective" anti-alcohol actions heretofore not observed with other benzodiazepine receptor inverse agonists. Together, these data support the hypothesis that many of the neuropharmacological effects of low to moderate doses of alcohol are mediated via augmentation of central GABAergic neurotransmission. Chronic administration or treatment with sedative/hypnotic drugs (barbiturates, benzodiazepines, ethanol) is generally associated with the development of pharmacodynamic tolerance. However, the mechanisms for this tolerance are obscure. Recently, we have shown that chronic barbiturate or ethanol administration to rats results in a 25-35% decrease in the apparent V_{max} of muscimol-stimulated $^{36}\text{Cl}^-$ uptake in cerebral cortical synaptoneurosomes. Following chronic ethanol administration, this "subsensitivity" in GABA receptor function is highly correlated to the blood ethanol level and with the development of the withdrawal syndrome.

Despite strong evidence that suggests benzodiazepine receptors mediate the anti-anxiety (anxiolytic) actions of the benzodiazepine (e.g., diazepam, chlordiazepoxide) and related compounds, the physiological function(s) of these sites is unclear. Several lines of evidence now suggest that these receptors may

play a role in the control of arousal and the central "stress" response. We have recently demonstrated that several stressors (forced ambient temperature swim, brief immersion in ice water, and food deprivation for the first three hours of the dark cycle) elicit a rapid and robust change in [^3H]benzodiazepine binding that is observed only in the presence of Eccles' permeable anions (e.g., chloride, iodide and bromide ions). These differences are manifest as an increase in the apparent affinity of [^3H]flunitrazepam, with no significant differences in the maximum number of binding sites (B_{max}) between the groups. Both an increase in the maximum enhancement of [^3H]flunitrazepam binding in response to optimum concentrations of halide ions (E_{max}) and an increased sensitivity to halide ions (reduced EC_{50}) were observed in response to stress. These results suggest that acute exposure to stress effects either the coupling of the chloride ionophore and benzodiazepine receptor, or that the chloride ionophore itself in some way modified. Further, the ability of muscimol to stimulate $^{36}\text{Cl}^-$ uptake *in vitro* is enhanced in synaptoneurosomes prepared from "stressed" rats when compared to stress-habituated controls. The effects of swim stress in potentiating muscimol-stimulated $^{36}\text{Cl}^-$ uptake were attenuated in adrenalectomized animals suggesting a "permissive" role for glucocorticoids in mediating this stress response. Recently, using an *in vivo* technique for labelling the benzodiazepine receptor (using [^3H]Ro15-1788) we have observed biphasic effects of swim stress on benzodiazepine receptor occupancy. Acute swim stress increases specific [^3H]Ro15-1788 binding immediately (≤ 1 hr) after stress whereas specific binding is decreased in many brain regions 24 hours after stress. Similar acute effects were observed after "defeat stress" another form of social stress. The possible role of adrenal steroid secretion in mediating the "delayed" effects of acute or chronic stress are currently being investigated.

Many membrane associated receptors have been shown to be sensitive to alterations in their lipid milieu. Changes in membrane lipids induced by activation of phospholipase A_2 (PLA_2) (an endogenous constituent of membranes) has been proposed as a physiologic mechanism for regulating receptor function. We have shown a differential sensitivity of "peripheral" and "central" benzodiazepine receptors to this enzyme. Phospholipase A_2 slightly increased the apparent affinity of the central benzodiazepine receptor ligands [^3H]flunitrazepam and [^3H]3-carboethoxy-B-carboline, with no concomitant change in the B_{max} of these ligands. In contrast, GABA enhanced [^3H]flunitrazepam was unaffected by PLA_2 . Both pyrazolopyridine and barbiturate enhanced [^3H]flunitrazepam binding were, however, reduced by very low (0.002 U/ml) concentrations of PLA_2 . Since both pyrazolopyridines and barbiturates bind to sites at or near the chloride ionophore, we examined the effects of PLA_2 on the specific chloride ionophore ligand [^{35}S]t-butylbicyclophosphorothionate (TBPS). It was found that PLA_2 inhibited [^{35}S]TBPS binding at the same concentrations needed to disrupt barbiturate and pyrazolopyridine enhanced [^3H]flunitrazepam binding. The inhibition of [^{35}S]TBPS binding by PLA_2 was manifest as a reduction in the B_{max} of this ligand with no change in the apparent affinity. PLA_2 was also found to reduce the apparent affinity of [^3H]Ro5-4864 to "peripheral" benzodiazepine receptors and the reduction in the apparent affinity of [^3H]Ro5-4864 was independent of the tissue source (e.g. the same reduction in apparent affinity was found in heart, kidney and brain membranes).

On screening extracts of various tissues for inhibitors of [3 H]Ro5-4864 binding we isolated and purified a 14 kDa protein "antralin" which was subsequently shown to have PLA₂ activity. Recently, "antralin" has been purified to homogeneity and sequenced using automatic Edmann analysis of tryptic fragments. Antralin was shown to be a member of the PLA₂ family based on its amino acid sequence. Substrate and Ca⁺⁺ dependency supported its PLA₂-like activity. Antibodies raised against antralin also cross-react with purified porcine pancreatic PLA₂. Studies on the possible physiological role of this protein in peripheral organs (stomach) and brain are currently underway.

Recently, the nucleotide sequence (along with the deduced amino acid sequence) of both the alpha and beta subunits of the bovine GABA_A receptor have been established through classic cloning techniques. Using this sequence we synthesized several oligonucleotide probes for both the alpha and beta subunit and screened a GT₁₁ cDNA library prepared from human brain. Several near full length cDNA clones for the alpha subunit have been isolated and have been used as probes to study the expression of receptor protein during or following various pharmacological and environmental conditions.

PUBLICATIONS:

1. Arora PK, Hanna EE, Paul SM, Skolnick P: Suppression of the immune response by benzodiazepine receptor inverse agonists. J Neuroimm 1987;15:1-9.
2. Mantione CR, Goldman ME, Martin B, Bolger GT, Lueddens HWM, Paul SM, Skolnick P: Purification and characterization of an endogenous protein modulator of radioligand binding to "peripheral-type" benzodiazepine receptors and dihydropyridine Ca²⁺-channel antagonist binding sites. Biochem Pharm 1988;37:339-47.
3. Miller LG, Greenblatt DJ, Abernethy DR, Friedman H, Luu MD, Paul SM, Shader RI: Kinetics, brain uptake, and receptor binding characteristics of flurazepam and its metabolites. Psychopharm 1988;94:386-391.
4. Moody EJ, Suzdak PD, Paul SM, Skolnick P: Inhalation anesthetics increase ³⁶Cl⁻ uptake into rat brain synaptoneurosomes. J Neurochem 1988;51(5):1386-93.
5. Morrow AL, Paul SM: Benzodiazepine enhancement of gamma-aminobutyric acid mediated chloride ion flux in rat brain synaptoneurosomes. J Neurochem 1988;50:302-6.
6. Morrow AL, Suzdak PD, Karanian JW, Paul SM: Chronic ethanol administration alters gamma-aminobutyric acid, pentobarbital and ethanol-mediated ³⁶Cl⁻ uptake in cerebral cortical synaptoneurosomes. J Pharm Exp Ther 1988;246(1):158-64.
7. Paul SM, Crawley JN, Skolnick P: The neurobiology of anxiety: the role of the GABA/benzodiazepine receptor complex. In: American Handbook of Psychiatry, 1987.

8. Schwartz RD, Skolnick P, Paul SM: Regulation of gamma aminobutyric acid/barbiturate receptor-gated chloride ion flux in brain vesicles by phospholipase A2: possible role of oxygen radicals. J Neurochem 1988;50(2):565-71.
9. Schwartz RD, Seale TW, Skolnick P, Paul SM: Differential seizure sensitivities to picrotoxinin in two inbred strains of mice (DBA/2J and BALB/c ByJ): Parallel changes in GABA receptor-mediated chloride flux and receptor binding. Brain Res, in press.
10. Skolnick P, Havoundjian H, Paul SM: Benzodiazepines and their receptors in anxiety disorders. In: Receptors and Ligands in Psychiatry and Neurology, Sen AK, Lee T (eds). Cambridge, Cambridge University Press, 1988;387-99.
11. Suzdak PD, Schwartz RD, Skolnick P, Paul SM: Alcohols stimulate gamma-aminobutyric acid receptor-mediated chloride uptake in brain vesicles: correlation with intoxication potency. Brain Res 1988;444:340-5.
12. Suzdak PD, Paul SM, Crawley JN: Effects of Ro15-4513 and other benzodiazepine receptor inverse agonists on alcohol-induced intoxication in the rat. J Pharm Exp Ther 1988;245(3):880-6.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02186-06 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Brain Recognition Sites for Stimulants and Antidepressants: Relationship to Pharmacological Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S.M. Paul	Chief	NS, NIMH
Other:	J.N. Crawley	Senior Staff Fellow	NS, NIMH
	E. Lestringant	Visiting Associate	NS, NIMH
	P. Skolnick	Pharmacologist	LBC, NIADDK

COOPERATING UNITS (if any)

Laboratory of Bioorganic Chemistry, NIADDK

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

3.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recognition sites for a variety of psychotherapeutic drugs have been identified in the central nervous system. Over the past several years we have attempted to identify recognition sites for other common psychotropic drugs including tricyclic antidepressants and the psychomotor stimulants, amphetamine and methylphenidate. In each case saturable, and stereospecific binding sites have been delineated; and for amphetamine and methylphenidate relatively good correlations have been observed between the affinities of a series of analogues in vitro and at least some of the pharmacological properties of these agents. Recent work has shown that the [3H](+)-amphetamine binding site in hypothalamic membranes is sensitive to circulating levels of blood glucose. Hypoglycemia decreases, and hyperglycemia increases, the number of [3H](+)-amphetamine binding sites in hypothalamic membranes respectively. Furthermore, these changes seemed to be coupled to the activity of (Na⁺ K⁺)(ATPase); and there is a good correlation between the changes in [3H](+)-amphetamine and [3H]-ouabain binding both in vivo and in vitro. More recent studies have shown that [3H]-mazindol a chemically unrelated anorectic/psychostimulant also can be used to label the [3H](+)-amphetamine cognition site and that there is a good correlation between the inhibition of [3H]-mazindol binding by a series of phenylethylamines and their anorectic effects in rats. These data suggest the existence of a membrane-bound receptor complex capable of "sensing" circulating glucose concentration and in regulating both glucostatic ingestive behavior and perhaps some aspects of the central regulation of energy metabolism. Recent work has demonstrated that genetically obese mice (ob/ob) have an abnormality in this system and fail respond to glucoprivic feeding signals. Over the past year, we have developed a method for measuring ouabain-sensitive ⁸⁶Rb uptake into synaptoneurosome and have used this method to measure "sodium pump" activity after treatment with anorectic drugs and in genetically obese rodents.

Project Description:Objectives:

1. To elucidate the mechanisms of action of important psychotropic drugs such as the psychomotor stimulants, anorectic agents and antidepressants.
2. To understand the neurochemical change associated with animal models of hyperphagia and obesity.

Methods Employed:

(See: 1984 Annual Report, pp 707-712 Project Number Z01 MH 02816-02 NS, Brain Recognition Sites for Stimulants and Antidepressants: Relationship to Pharmacological Activity.)

Major Findings:

High affinity, stereospecific binding sites for [3H] mazindol have been previously described in rodent brain. The highest density of these sites are found in the synaptosomal fraction of brainstem and hypothalamus. A good correlation has been demonstrated between the ability of a series of phenylethylamine derivatives in displacing [3H] mazindol binding and their potencies as anorectic agents. These observations suggest that the [3H]-mazindol binding site (anorectic drug recognition site, ADRS) may be involved in the appetite suppressant actions of chemically unrelated anorectic agents. The ADRS has also been studied in genetically obese mice (ob/ob). In these animals the density of hypothalamic ADRS is greater than in lean litter mates. Furthermore, food deprivation of rats (24-72 hours) results in a dramatic (35-50%) reduction in the density of hypothalamic ADRS. Refeeding food-deprived animals for a four hour period (or allowing access to a 10% glucose solution) results in a return of ADRS density to control values. These data suggest that the ADRS may be intimately involved in the regulation of feeding behavior. In more recent experiments the changes in ADRS during food deprivation and refeeding have been localized to discrete hypothalamic and brainstem nuclei (paraventricular nucleus and nucleus tractus solitarius).

Since the ADRS in hypothalamus is decreased following 24 hours of food deprivation (30% reduction in (Bmax), and the site density restored to control levels if the animals are permitted to refeed for four hours we have examined the factors responsible for this rapid modulation in site number. Good correlations between circulating glucose concentration and the changes in ADRS during food deprivation and refeeding suggest that glucose (or a metabolite) regulate the density of ADRS.

Injection of 2-deoxy-D-glucose also elicits a significant increase in ADRS in the hypothalamus and brainstem of rats and mice. However, this treatment did not alter ADRS in other brain regions. Further, injection of 2-deoxy-D-glucose elicited increases in ADRS density that were again confined to the PVN and NTS. Interestingly, if animals are permitted access to food during the four hour interval following injection of 2-deoxy-D-glucose, no increase in ADRS is observed. These observations suggest that the ADRS in hypothalamus are coupled to glucose utilization and "hunger". The good correlation previously reported

between the ability of a number of phenethylamines to inhibit ADRS and their potencies as anorectics may thus link the anorectic actions of phenethylamines with their ability to effect glucose-responsive neurons in the hypothalamus. Although the density of ADRS in hypothalamus is increased in genetically obese (ob/ob) mice the latter fail to increase food intake following administration of 2-deoxy-D-glucose. Further no further increase in hypothalamic ADRS is observed in ob/ob mice following 2-deoxy-D-glucose administration.

In related experiments the regulation of ADRS in hypothalamic tissue slices in vitro have confirmed that glucose plays a major role in determining the density of ADRS. Moreover, the ability of glucose to stimulate ADRS in hypothalamic slices in vitro is blocked by ouabain and correlated with similar increases in [3H]ouabain binding and Na+K+ ATPase activity. These data suggest a close functional coupling between the ADRS and Na+K+ ATPase. In related studies we have found that the genetically obese mouse has not only an increased density of ADRS, but of [3H]ouabain binding and Na ATPase activity in several brain regions. Together, with the lack of hyperphagic response to 2-deoxy-D-glucose, these data suggest that the genetically obese mouse has an altered glucostatic satiety signal.

Previous studies in our laboratory have demonstrated the presence of high affinity, stereospecific binding sites for [3H] (\pm) threo-methylphenidate in the striatum and brainstem of the rat. Subsequent studies demonstrated that the binding of [3H]-methylphenidate is localized to synaptosomes, and that the binding is dependent on the presence of sodium. Intraventricular administration of 6-hydroxydopamine or medial forebrain bundle lesions results in a significant loss of [3H]-methylphenidate binding in striatum which is highly correlated with a loss in the capacity of this tissue to take up [3H]-dopamine. Structure-activity studies suggest that this site is associated with a dopamine transport system since a good correlation ($r=0.88$, $p<0.001$) was found between the potencies of a series of compounds to inhibit [3H]-dopamine uptake and [3H]-methylphenidate binding. These findings suggest that the methylphenidate binding site may be part of a dopamine "transporter". In a related series of experiments several diphenyl-substituted piperazines (GBR-12935, GBR-12921) have been tested for their selectivity in inhibiting dopamine uptake. The marked specificity of these compounds in inhibiting dopamine uptake has prompted the radioactive labeling of GBR-12935. [3H]-GBR-12935 appears to be a "super high affinity" ligand for the dopamine uptake site and may be useful for in vivo imaging of dopamine-containing neurons. Studies with postmortem human brain have further documented the association of [3H] GBR-12935 binding to dopamine neurons since we observed significant decreases in [3H] GBR-12935 binding in striatal tissue from Parkinson's patients.

Significance to Biomedical Research and Program of the Institute:

All of the drugs under investigation have important psychotropic and behavioral actions and are either of therapeutic benefit or reliably mimic various behavioral states. Thus, an understanding of their mechanisms of actions should be of value to understanding the behavioral and psychopathological states responsive to treatment with these agents.

Proposed Course:

Studies will continue on the various recognition sites described above to more fully elucidate their pharmacological as well as physiological significance. A major emphasis will be placed on defining the alterations in ADRS density that occur in vivo during various manipulations of "appetite" and "satiety", in order to test the hypothesis that these sites are coupled to a physiological mechanism regulating food intake (particularly carbohydrate intake) in animals. The relationship between ADRS and the neuronal form of Na⁺K⁺ ATPase will also be investigated using both binding, enzyme assay, and ⁸⁶Rb flux measurements. Emphasis will be placed on whether these binding sites label some novel postsynaptic effector system and whether conventional neurotransmitters such as dopamine and serotonin regulate these sites *in vitro*.

Publications

1. Hauger, R.L., Skolnick, P. and Paul, S.M.: Brain recognition sites for typical and atypical antidepressants. In: Advances in Human Psychopharmacology, Vol. IV, G.D. Burrows and J.S. Werry (Eds.), Pergamon Press, New York, in press.
2. Skolnick, P., Schweri, M.M., Rafferty, M.F., Rice, K.C., Janowsky, A.J. and Paul, S.M. [3H]-threo-(±)-Methylphenidate binding in neuronal dopamine uptake sites in corpus striatum: correlation with the stimulant properties of ritalinic acid esters. J. Neurochem, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02340-03 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Clinical

Studies of Gaucher Disease and Other Neurogenetic Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.I. Ginns, Head, Molecular Neurogenetics Unit, NS, NIMH

Others:	W. Eliason	Guest Researcher	NS, NIMH
	M. LaMarca	Microbiologist	NS, NIMH
	B. Martin	Visiting Scientist	NS, NIMH
	B. Martin	Guest Researcher	NS, NIMH
	E. Sidransky	Guest Researcher	NS, NIMH
	B. Stubblefield	Biologist	NS, NIMH
	S. Winfield	Microbiologist	NS, NIMH

COOPERATING UNITS (if any)

Human Genetics Branch, National Institute of Child Health and Human Development;
 Interinstitute Genetics Program, NIH; Arthritis Service, Hospital for Joint
 Diseases, New York, NY

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Molecular Neurogenetics Unit, Section on Preclinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.6

PROFESSIONAL:

0.7

OTHER:

1.9

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The clinical study of human genetic disorders that affect the nervous system provides foundation for the development of improved diagnostic techniques and strategies for therapy. This goal is greatly facilitated by having a comprehensive knowledge of the biochemistry and clinical heterogeneity of the disorder. Gaucher disease, the most common sphingolipidosis, has a high priority as a model for gaining insight into this group of neurogenetic disorders because of the occurrence of both neuronopathic and non-neuronopathic phenotypes as well as the broad spectrum of clinical diversity within the major types of the disorder. Once the pathophysiologic mechanisms of systemic involvement are understood, the therapy of nervous system dysfunction may be more rationally approached. Basic research on glucocerebrosidase, the enzyme deficient in Gaucher disease, has generated a more detailed understanding of the structure, biosynthesis, intracellular routing, and turnover of the enzyme. These studies will complement other studies within our branch focusing on the investigation of the potential and efficacy of gene transfer as a therapeutic approach. The use of recombinant DNA technologies to develop an appropriate animal model and to produce large amounts of both normal and mutant human proteins is being pursued.

OTHERS:

K. Maysak	Guest Researcher	NS, NIMH
Various	Genetic Fellows	IGP, NIH
J. Sidbury	Chief, Section on Human Biochem Genetics	HGB, NICHD
S. Stuchin	Chief, Arthritis Service	HJD

PROJECT DESCRIPTION

Biochemical: From our study of the molecular biology of Gaucher disease we plan to: purify mutant enzymes; characterize the primary amino acid sequences and post-translational processing of the mutant enzymes; correlate the structural mutations of the protein with the observed clinical heterogeneity; characterize the structure, organization, and regulation of expression of the normal mutant glucocerebrosidase genes; investigate the production of large quantities of protein using recombinant DNA methodologies; develop diagnostically useful recombinant DNA tests (i.e., RFLPs); develop an animal model of Gaucher disease; and evaluate the potential of somatic cell gene therapy for Gaucher disease.

Clinical: Using Gaucher disease as a prototype of inherited disorders having both neurologic and non-neurologic phenotypes, clinical evaluations are undertaken in an attempt to correlate the clinical heterogeneity with both biochemical and genetic data. The role of the macrophage in pathogenesis of the numerous clinical manifestations of this disorder will be studied. Specific approaches to therapeutic intervention for Gaucher disease focus on the hypothesis that this disorder is a macrophage disorder. The involvement of hematopoietic derived cells in the pathogenesis of this disorder is crucial to the applicability of somatic cell gene therapy as a potential therapeutic strategy.

METHODS EMPLOYED

(SEE: 1987 Annual Report, Project Number Z01 MH 02340-02 NS, pp 443-445)

MAJOR FINDINGS

1. Recombinant normal human glucocerebrosidase was purified on a pilot scale.
2. Antibodies were used for studying the glycosylation of recombinant glucocerebrosidase using Western blot analysis.
3. Carbohydrate analysis and amino acid sequencing were performed on recombinant glucocerebrosidase using protein immobilized on teflon membranes.
4. The post-translation processing of glucocerebrosidase in Type 1, 2 and 3 Gaucher disease was further characterized.
5. The normal and pseudogene loci for the glucocerebrosidase gene continue to be characterized.
6. Several single base mutations have been identified in patient's DNA.

7. All the exons splice junctions and flanking regions of a genomic clone from a type 1 patient were sequenced and a single base change occurring in high frequency among non-neuronopathic phenotype (type 1) of Gaucher disease was identified.
8. Useful diagnostic test based on hybridization of oligonucleotide probes, for the identification of mutations in Gaucher disease continue to be developed.
9. The expression by DNA mediated gene transfer of active human glucocerebrosidase in heterologous mammalian host cell lines was extended to include high-level production in the baculovirus expression system.
10. The correction of the enzyme deficiency in type 2 Gaucher fibroblasts in culture by retroviral mediated gene transfer using cDNA retroviral constructs is being followed by experiments with genomic constructs.
11. The spectrum of symptoms of patients having Gaucher disease was further studied and correlated with RFLP analysis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

Gaucher disease is useful as a prototype disorder for furthering our understanding of the mechanisms responsible for the clinical heterogeneity seen within many of the neurogenetic disorders. It is the most common sphingolipidoses and many patients could benefit from the development of therapy. The techniques and information obtained from the study of the protein processing and gene in Gaucher disease should be useful and helpful to formulating strategies for understanding the biochemical and genetic bases of other neuropsychiatric disorders.

PROPOSED COURSE

Patients having type 1, 2 or 3 Gaucher disease will be studied to further define the biochemical and genetic mechanisms responsible for the clinical heterogeneity within this disorder. The involvement of hematopoietic stem cell derived macrophages in the pathogenesis of symptoms makes type 1 Gaucher disease an attractive candidate for somatic cell gene therapy.

PUBLICATIONS

Barranger JA, Ginns EI: Glucosylceramide lipidoses: Gaucher disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The Metabolic Basis of Inherited Disease. New York: McGraw-Hill (in press).

van Dongen JM, Willemsen R, Ginns EI, Sips HJ, Tager JM, Barranger JA, Reuser AJJ. Subcellular localization of soluble and membrane-bound lysosomal enzymes in I-cell fibroblasts: A comparative immunocytochemical study, Eur J Cell Biol (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02341-03 NS
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Correction of Inherited Enzyme Deficiencies by Gene Transfer		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: E.I. Ginns, Head, Molecular Neurogenetics Unit, NS, NIMH		
Others: B. Martin Visiting Scientist E. Sidransky Guest Researcher B. Stubblefield Biologist M. LaMarca Guest Researcher S. Winfield Microbiologist B. Martin Guest Researcher W. Eliason Guest Researcher		NS, NIMH NS, NIMH NS, NIMH NS, NIMH NS, NIMH NS, NIMH NS, NIMH
COOPERATING UNITS (if any) Center for Cancer Research, MIT and Whitehead Institute for Biomedical Research, Cambridge, MA		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Molecular Neurogenetics Unit, Section on Preclinical Studies		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.8	PROFESSIONAL: 0.7	OTHER: 1.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The isolation and characterization of normal and abnormal proteins in the genetic disorders affecting the nervous system has permitted the isolation of <u>cDNA</u> and <u>genomic DNA</u> that can be used to investigate the <u>correction</u> of inherited protein deficiencies using <u>recombinant DNA techniques</u>, specifically <u>somatic cell gene transfer</u>. Particularly suited for initial attempts at gene therapy are those disorders (such as <u>Gaucher disease</u>, the most common <u>sphingolipidosis</u>) in which the manifestations of the disorder are due to abnormalities of <u>hematopoietic cells</u>, in this case, the <u>macrophage</u>. In this instance the transfer of normal genes to <u>bone marrow progenitor</u> cells is a rationale therapeutic approach. Using the <u>lysosomal</u> disorder Gaucher disease as a model, we have been successful in utilizing <u>retroviral vectors</u> to transfer and express human glucocerebrosidase in host <u>mouse</u> and Gaucher cell lines. The complete correction of glucocerebrosidase activity in <u>Gaucher fibroblasts</u> in <u>culture</u> has provided the impetus for evaluation of <u>retroviral mediated somatic cell gene transfer</u> of the <u>glucocerebrosidase gene</u> into mice by <u>bone marrow transplantation</u>. The initial goal of this research is the application of these recombinant DNA therapeutic strategies to the non-neuronopathic phenotypes. Animal models are being developed using recombinant technologies. When our understanding of the pathogenetic mechanisms of inherited <u>neurological</u> and <u>psychiatric</u> diseases improves and when <u>retroviral-mediated expression</u> of genes in specific tissues and cells become more predictable, we can begin to investigate the potential usefulness of <u>gene therapy</u> for treatment of selected disorders affecting the <u>nervous system</u>. </p>		

OTHERS:

D. Wright	Guest Researcher	NS, NIMH
K. Maysak	Guest Researcher	NS, NIMH
R. Mulligan		MIT
B. Guild		MIT
E. Dzierzak		MIT
J. LeGros	Guest Researcher	NS, NIMH

PROJECT DESCRIPTION

The isolation of cDNA and genomic DNA encoding specific proteins involved in neurogenetic disorders permits the application of recombinant DNA technologies as therapeutic approaches to the correction of these inherited enzyme deficiencies. Using the lysosomal storage disorder Gaucher disease as a prototype we are investigating the efficacy of retroviral mediated gene transfer, first applied in tissue culture and then in small animals.

METHODS EMPLOYED

(SEE: 1987 Annual Report, Project Number Z01 MH 02341-02 NS, pp 447-449)

MAJOR FINDINGS

1. Normal and mutant complementary DNA and genomic DNA clones encoding human glucocerebrosidase continue to be isolated and sequenced.
2. Mapping of both the functional and genomic loci pseudogene for human glucocerebrosidase continues.
3. Active and mutant human glucocerebrosidase has been transferred to both mouse and monkey cell lines using eukaryotic shuttle vectors.
4. The enzyme deficiency in Gaucher fibroblasts in tissue culture has been corrected by transfer of the normal human glucocerebrosidase cDNA to these cells.
5. Recombinant retrovirus containing human glucocerebrosidase cDNA has been used to infect mouse bone marrow cells and obtain reconstituted mice having provirus in their blood cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

The isolation of the cDNA and genomic DNA for human glucocerebrosidase and the transfer of this gene by retroviral vectors suggests that such recombinant DNA approaches may be useful therapeutic strategies for Gaucher disease and other selected genetic disorders.

PROPOSED COURSE

The project initially focuses on the DNA mediated-transfer of normal, proteins to rodent and human cell lines in culture. Once the efficacy of gene transfer (particularly retroviral-mediated) is demonstrated, the approach will be applied to appropriate animal models and then to human subjects with specific inherited disorders (such as Gaucher disease).

PUBLICATIONS

Martin BM, Tsuji S, LaMarca ME, Maysak K, Eliason W, Ginns EI. Molecular biology of Gaucher disease: Therapeutic strategies utilizing recombinant DNA technologies, in NATO Advanced Research Workshop and Inserm Symposium: Lipid Storage Disorders (Biological and Medical Aspects), Toulouse 1988, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02342-03 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Regulation within the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.I. Ginns, Head, Molecular Neurogenetics Unit, NS, NIMH

Others:	B. Martin	Visiting Scientist	NS, NIMH
	S. Winfield	Microbiologist	NS, NIMH
	P. Marangos	Unit on Neurochemistry	BPB, NIMH
	D. Schmeckel	Neurology Department	VAMC
	J. Polak	Resident Scientist	RPMS
	J. Hozier	Medical Genetics	FIT

COOPERATING UNITS (if any)

Unit on Neurochemistry, Biological Psychiatry Branch, NIMH; Veterans Administration Medical Center, Durham, NC; Royal Post-Graduate Medical School, London, England; Florida Institute of Technology, Melbourne, FL

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Molecular Neurogenetics Unit, Section on Preclinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.4

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We approached the cell-specific and developmentally regulated expression of proteins within the nervous system using the neuron specific (NSE) and non-neuronal (NNE) enolase isozymes as a model. Human brain cDNA and genomic DNA libraries were constructed so that the genes for these and other brain specific proteins could be isolated and characterized. Using both antibodies and oligonucleotide probes, cDNAs for both human NSE and NNE have been isolated and sequenced. Employing unique regions of these cDNA clones as probes, the developmentally and cell-specific regulated appearance of mRNA for each of these proteins can be investigated using in-situ hybridization. The human chromosome loci for each of these isozymes will be identified. In addition, the isolation of human genomic clones for each of these proteins should provide information on the regulation of expression of neuron and glial specific proteins during cell differentiation of the human nervous system in normal and disease states. The normal specificity of NSE for neural derived cell lines and the availability of specific DNA probes for NSE should provide a useful approach to the characterization of neural derived normal and tumor cell lineages.

PROJECT DESCRIPTION

The enolase isozymes will be used as a model for studying the transcriptional and translational control of developmentally regulated genes within the human nervous system. The genomic organization and regulation of expression of these genes will be investigated.

METHODS EMPLOYED

(SEE: 1987 Annual Report, Project Number Z01 MH 02342-02 NS, pp 451-452)

MAJOR FINDINGS

1. Region specific human brain cDNA and genomic DNA libraries have been constructed.
2. Complementary DNA clones for human neuron specific and non-neuronal enolases have been isolated and sequenced.
3. Specific cDNA probes for NSE and NNE have been demonstrated to be useful for high resolution in-situ chromosome localization and tissue hybridization studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

A description of the transcriptional and translational control mechanisms for neuron specific and non-neuronal enolases should provide a more detailed understanding of the mechanisms involved in regulation of gene expression in neurons and glia within the human nervous system during development and differentiation.

PROPOSED COURSE

The project will focus on the description of the genomic organization and control mechanisms of expression of NSE and NNE genes. The involvement of mutations in the genes for these proteins in the pathogenesis of neuropsychiatric disorders will be investigated.

PUBLICATION

Schmechel DE, Marangos PJ, Martin BM, Winfield S, Burkhart DS, Roses AD, Ginns EI. Localization of neuron-specific enolase (NSE) mRNA in human brain, *Neurosci Lett* 1987;76:233-8.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02343-03 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Inherited Neurologic and Psychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: E.I. Ginns, Head, Molecular Neurogenetics Unit, NS, NIMH

Others:	S.M. Paul	Acting Chief, Sect Molecular Pharmacology	NS, NIMH
	D. Pickar	Chief, Sect Clinical Studies	NS, NIMH
	J. Kelsoe	Medical Staff Fellow	NS, NIMH
	B. Martin	Visiting Scientist	NS, NIMH
	S. Stubblefield	Biologist	NS, NIMH
	S. Winfield	Microbiologist	NS, NIMH
	D. Klein	Chief, Sect Developmental Neurobiology	NICHHD

COOPERATING UNITS (if any)

Pediatrics Department, Johns Hopkins School of Medicine, Baltimore, MD; Florida Institute of Technology, Melbourne, FL; Dept of Anatomy & Neurobiology, Washington Univ School of Medicine

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Molecular Neurogenetics Unit, Section on Preclinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

0.9

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We approached the characterization of the mutations responsible for inherited neurologic or psychiatric disorders by studying the gene organization of specific proteins that might have a role in the pathogenesis of the clinical manifestations. Using the inherited lysosomal storage disorders, Gaucher disease and Fabry disease, as models, we demonstrated that the phenotypic heterogeneity seen within these inherited disorders is a consequence of different mutations, each affecting protein activity and influencing the processing, compartmentalization and/or stability of the protein. Restriction fragment length polymorphisms (RFLPs) have been identified that are useful for the identification of mutations in Gaucher disease that frequently occur in both non-neuronopathic and neuronopathic phenotypes. Northern blot analysis provides further details of the structure of the normal and mutant genes. The molecular mechanisms leading to nervous system involvement in these disorders have also been investigated. The results of this research should provide a more rational foundation for the diagnosis and formulation of therapeutic strategies for these inherited disorders. Similar approaches are being used to investigate the involvement of candidate genes in bipolar illness and other psychiatric disorders. Recombinant DNA techniques have been used to elucidate the structure of genes for the proteins (enzymes and receptors) that may be involved in neuropsychiatric disorders. Genes specific for neurotransmitter biosynthesis (for example, human tyrosine hydroxylase and tryptophan hydroxylase) and receptors (for example, human GABA receptor) have been isolated. Comparison of normal gene sequence to the gene sequence in Amish bipolar patients is in progress. Recombinant DNA expression systems have been used to produce large amounts of these enzymes of receptors tyrosine hydroxylase isozyme for structural and biochemical studies.

OTHERS

L. Morgenstern	Guest Researcher	NS, NIMH
J. Hozier	Medical Genetics	FIT
K. O'Malley	Anatomy & Neurobiology	Wash Univ Sch Med

PROJECT DESCRIPTION

The observed differences in presentation of neurogenetic disorders may be a consequence of multiple allelic mutations or the involvement of more than a single gene. The understanding of the mechanisms of this phenotypic heterogeneity will be derived from genetic and biochemical analyses. Recombinant DNA techniques are used to isolate and characterize the genes for specific proteins. The study of mutations should elucidate the structural abnormalities and consequences of the abnormal biosynthesis and post-translational processing of the mutant proteins. The identification of RFLPs associated with clinical manifestations is studied using cDNA and genomic DNA probes. The comparison of gene expression for proteins in neural and non-neural tissues should extend our understanding of protein regulation.

METHODS EMPLOYED

(SEE: 1987 Annual Report, Project Number Z01 MH 02343-02 NS, pp 453-455)

MAJOR FINDINGS

1. Complementary DNA and genomic libraries were constructed from human brain tissue.
2. Human cDNA clones encoding active tyrosine hydroxylase, tryptophan hydroxylase and GABA receptor, have been isolated and sequenced.
3. Mutations have been identified within the coding regions of a Gaucher genomic DNA clones.
4. Chromosome 11 specific probes (globin, harvey ras, tyrosine hydroxylase) have been obtained for RFLP analysis of Amish patient's having bipolar affective illness.
5. The normal gene for human tyrosine hydroxylase was isolated and sequenced.
6. Active recombinant human tyrosine hydroxylase has been produced.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

The description of the molecular basis for neuropsychiatric disorders should provide a more rational basis for the development of diagnostic and therapeutic strategies.

PROPOSED COURSE

The project will focus on the biochemistry and genetics of these disorders to

obtain a more complete understanding of the mechanisms responsible for the clinical manifestations of these inherited disorders. This information will be used to develop diagnostic and therapeutic strategies.

PUBLICATIONS

Ginns EI. Application of immunoblotting to the study of the molecular genetics of inherited metabolic disorders. In: Bjerrum OJ and Heegaard NHH, eds. Handbook of Immunoblotting. Florida: CRC Press, 1988, Vol 2, Chap 9.5.

O'Malley KL, Anhalt M, Martin BM, Kelsoe JR, Winfield SL, Ginns EI. Isolation and characterization of the human tyrosine hydroxylase gene: Identification of 5' alternative splice sites responsible for multiple mRNAs, Biochemistry (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02344-03 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychiatric Disorders: Protein Structure-Activity Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. Martin, Visiting Scientist, NS, NIMH

Others: E.I. Ginns	Head, Molecular Neurogenetics Unit	NS, NIMH
W. Eliason	Guest Researcher	NS, NIMH
K. Maysak	Guest Researcher	NS, NIMH
L. Possani	Free University of Mexico, Mexico	

COOPERATING UNITS (if any)

Free University of Mexico, Mexico

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Molecular Neurogenetics Unit, Section on Preclinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.1

PROFESSIONAL:

0.7

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research is part of an effort to better understand the molecular mechanisms underlying human nervous system development and function, as well as the pathogenesis of certain neurogenetic disorders. Our studies have focused on structural and active site properties of the human neuron specific proteins, lysosomal hydrolases (glucocerebro-sidase and alpha-galactosidase A), other enzymes (particularly those peptides and proteins that interact with excitable membranes), receptors and venom toxins. Proteins are purified from both human and animal tissues using affinity chromatography, electrophoretic separation, and high performance liquid chromatography. Amino acid sequence of glucocerebrosidase, antralin and major portions of sequences for the neuronal and non-neuronal enolases, venom toxins. Beta galactosidase and alpha-galactosidase A have been obtained using microsequencing techniques. Peptide maps of both normal and mutant proteins are generated using chemical (cyanogen bromide) and enzymatic (trypsin, thermolysin, V8 protease) cleavage. The identification of carbohydrate attachment sites, sulfhydryl residues, and intra-chain disulfide residues is used to predict protein structure. Alkylating agents and enzyme inhibitors are used to define active sites. From the primary protein sequence, hydrophobic and hydrophilic domains of the protein are identified.

Information obtained from these protein structure studies permits the design of oligonucleotides and peptides that are synthesized for collaborative research involving antibody production, cDNA cloning, DNA sequence analysis and in vitro mutagenesis.

PROJECT DESCRIPTION

A goal of this project includes the identification of primary and tertiary structure of the proteins. Once these aspects of protein structure are elucidated, a three-dimensional model can be constructed using the secondary structure data obtained from computer modeling. This information will be useful in defining the hydrophilic and membrane domains of the protein. Active sites are identified using sulfhydryl reagents and specific inhibitors and activators. This information is used to design synthetic oligonucleotides and peptides for collaborative research.

METHODS EMPLOYED

(SEE: 1987 Annual Report, Project Number Z01 MH 02344-02 NS, pp 457-458)

MAJOR FINDINGS

1. The amino acid sequence of analysis of human lysosomal enzymes as well as identification of carbohydrate attachment sites and disulfide bridges continues.
2. Biochemical characterization, including partial amino acid sequence, of a novel calcium binding protein from snake venom and several potassium channel blocking toxins from scorpion.
3. Determination of the amino acid sequence of antralin, an unusual phospholipase A2 from rat stomach.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

The neuron specific proteins are used as model systems to investigate the developmentally controlled expression of specific proteins within the nervous system. The lysosomal hydrolases are used as prototypes for understanding the phenotypic heterogeneity disorders affecting the nervous system. Studies of venom toxins should provide information on the structure of ion channels within the nervous system. Together these studies further our understanding of the structure-function relationships of nervous system proteins.

PROPOSED COURSE

The project will focus on the proteins described above as well as other proteins involved in lysosomal and neuropsychiatric disorders.

PUBLICATIONS

Alagon AC, Guzman HS, Martin BM, Ramirez AN, Carbone E, Possani LD. Isolation and characterization of two toxins from the Mexican scorpion *centruroides limpidus karsch*. *Comp Biochem Physiol* 1988;89B:153-61.

Mussar KJ, Murray GJ, Martin BM, Viswanatha T. Rapid chromatographic assay procedure for peptide-n-glycosidase activity. *J Chromat* 1987;408:378-84.

Zasloff M, Martin BM, Chen HC. Antimicrobial polypeptides from *X. laevis* skin: sequencing and synthesis of the Magainin family. Proc Natl Acad Sci (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02181-06 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Pickar Chief, Section on Clinical Studies NS, NIMH

Others:	S.M. Paul	Chief	NS, NIMH
	J.R. Kelsoe	Medical Staff Fellow	NS, NIMH
	P.E. Konicki	Medical Staff Fellow	NS, NIMH
	R.E. Litman	Medical Staff Fellow	NS, NIMH
	R.R. Owen	Medical Staff Fellow	NS, NIMH
	M.H. Rapaport	Medical Staff Fellow	NS, NIMH
	J.L. Schreiber	Social Worker	NS, NIMH

COOPERATING UNITS (if any)

Laboratory of Cerebral Metabolism, NIMH; Clinical Brain Disorders Branch, St. Elizabeths Hospital, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

7.0

PROFESSIONAL:

5.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project studies the psychobiology of schizophrenia and attempts to develop improved strategies for its treatment. One goal is the understanding of the mechanism of action of neuroleptic drugs. We have observed that neuroleptic-induced time-dependent decrease in levels of plasma homovanillic acid (HVA), a major dopamine metabolite, correlates with antipsychotic drug response, suggesting that slow to develop changes in dopamine turnover may underlie the antipsychotic action of neuroleptics. This clinically relevant dopamine marker is further studied using a strategy in which peripherally derived HVA is reduced by the administration of debrisoquin, a MAO inhibitor which does not enter the CNS. Longitudinal studies of the atypical neuroleptic, clozapine, have begun in in/ and outpatient settings. This drug holds promise for enhancing the therapeutic effectiveness of neuroleptic treatment of schizophrenia and our studies are geared toward delineating fundamental mechanisms involved in its unique efficacy. In a recently completed study using magnetic resonance imaging (MRI) performed in collaboration with the Clinical Brain Disorders Branch, NIMH, compelling evidence was gained which supports enlargement of lateral and 3rd ventricular volumes in schizophrenic patients. A follow-up study of previous inpatients from our program has recently been completed and holds promise for delineating biological correlates of outcome in schizophrenia. Further studies of outcome are in progress. The development of extended pedigrees of families with high prevalence of schizophrenia is underway with the goal of establishing genetic linkage by RFLP techniques to the disease transmission. Collaboration with the NIMH positron emission tomography (PET) program focuses on the mechanism of action of typical and atypical neuroleptics using glucose utilization studies and on the development of new PET ligands.

OTHERS

C.N. Pato	NRSA Fellow	NS, NIMH
R.M. Cohen	Chief, Clinical Brain Imaging Section	LCM, NIMH
D. Weinberger	Chief, Clinical Brain Disorders Branch	NIMH

PROJECT DESCRIPTION

This project is part of the research program of the Section on Clinical Studies of the Clinical Neuroscience Branch. This section conducts clinical research on the 4-East Nursing Unit and the ACRF of the Clinical Center.

Despite enormous research and clinical efforts to alleviate symptoms of schizophrenia, the group of drugs known as neuroleptics have remained the principal pharmacologic agents for treatment of this illness. Moreover, the effects of neuroleptics on CNS dopamine systems also represent the cornerstone for the dopamine hypothesis of schizophrenia.

We have performed longitudinal clinical studies in which neuroleptic-induced alterations in dopamine function are assessed using levels of plasma homovanillic acid (HVA). Patterns of neuroleptic-induced alteration in plasma HVA level are examined as predictors of clinical response. Plasma HVA levels are also examined with regard to their relationship to CSF metabolite levels. Administration of the MAO-A inhibitor, debrisoquin, is used to enhance the relative contribution of CNS derived HVA to levels which circulate in plasma. Positron emission tomographic (PET) studies in which regional brain glucose utilization is examined in response to specific psychological tests before and during treatment with typical and atypical neuroleptics is in progress. These studies are used to further elucidate the mechanism underlying neuroleptic effects as well as to study pathophysiologic mechanisms in schizophrenia in general. These functional brain imaging studies are performed in tandem with structural brain imaging methods using computerized tomography (CT) and magnetic resonance imaging (MRI) techniques.

We have begun investigations into the clinical efficacy and biological mechanisms associated with the atypical neuroleptic, clozapine. We are currently applying our plasma HVA techniques as well as the use of functional brain imaging to study mechanisms of clozapine action. These studies add to our previous work investigating adjuncts to neuroleptic treatment in chronically ill patients with schizophrenia.

Two new methodologies have recently been developed and applied to our studies of schizophrenic patients. Studies of abnormalities in smooth pursuit eye tracking and in saccadic eye movements are underway in our inpatient and outpatient programs. These investigations are summarized under Project Number Z01 MH 02345-01 NS. Another developing research area is the possible involvement of autoimmune mechanisms to the pathophysiology of schizophrenia. The methodology and findings to date in these studies are summarized in Project Number Z01 MH 02346-01 NS.

Outpatient strategies in schizophrenia research are increasingly utilized by the Section. An initial follow-up study of patients who had been discharged from our research program, from 2-12 years previously, is now completed. The success of

this study has prompted further investigations into biological predictors of moderate- to long-term outcome in schizophrenia. Outpatients also participate in a study of the atypical neuroleptic, clozapine, providing an additional cohort of patients in whom clozapine action can be studied. In aggregate, the outpatient research facility is adding significant momentum to the Section's biological studies of schizophrenia.

METHODOLOGY

(See: 1987 Annual Report, pp 461-467, Project Number Z01 MH 02181-05 NS, Neurobiology of Schizophrenia)

The atypical neuroleptic, clozapine, is being administered under double-blind conditions to patients on the 4-East Nursing Unit following a 5-week neuroleptic-free period. Longitudinal strategies in which levels of plasma HVA, MHPG and CSF metabolites are sampled are utilized. Clinical and biological effects of clozapine are compared with responses observed in the same patients during typical neuroleptic treatment. Patients who respond favorably to clozapine will be followed as outpatients.

Eye tracking and immunologic studies are performed per the methodology described in Project Numbers Z01 MH 02345-01 NS and Z01 MH 02346-01 NS, respectively.

In collaboration with the Unit on Molecular Neurogenetics we have developed pedigrees of families with high prevalence of schizophrenia. Diagnostic procedures utilizing SADS-L structured interviews and blind concordance ratings are performed for well and ill members of these families. Cell lines from these families are immortalized for subsequent linkage analysis.

MAJOR FINDINGS

1. Expanded data support our initial findings that neuroleptic treatment produces time-dependent decreases in plasma levels of the dopamine metabolite, HVA, and that neuroleptic-induced changes in levels of plasma HVA correlate with clinical response. Preliminary data using the debrisoquin strategy show similar time-dependent decreases in plasma HVA levels, supporting the notion that this pattern reflects changes in CNS dopamine systems. These data support the hypothesis that neuroleptic drugs produce slowly developing alterations in presynaptic dopamine activity. The applicability of this plasma metabolite model for clinical setting is under study in the outpatient program.

In studies of CSF metabolites in schizophrenic patients, we have observed that lower levels of CSF HVA are associated with more highly symptomatic patients. These data contrast with the positive relationship between levels of plasma HVA and symptomatology. Multivaried analysis suggests that plasma and CSF HVA levels may predict differing aspects of the symptomatology of schizophrenia. Differences between CSF and plasma HVA include their response to neuroleptic treatment. In contrast to the neuroleptic-induced reduction seen in plasma HVA, CSF HVA levels tend to increase, and remain increased, during neuroleptic treatment. This discrepancy may relate to particularly prominent effects of mesocortical dopamine neurons in determining levels of CSF HVA.

2. Extension of the implications of positive and negative symptoms in schizophrenia has been gained from our follow-up study. We have observed that outcome (levels of functioning at work and in personal life) are correlated with both positive and negative symptoms; moreover, each symptom group predicts different aspects of outcome. We have observed significant correlations between ratings of negative symptoms and performance on Wisconsin Card Sort (WCS), a psychological test which reflects frontal cortical function. Thus, despite earlier findings suggesting that both positive and negative symptoms show significant overlap to neuroleptic treatment, emerging data indicates that these symptoms may not simply be mirror reflections but rather reveal differing pathophysiologic determinants of schizophrenia.

3. We find no consistent relationship between negative and positive symptom profiles and CT abnormalities including generalized and frontal cortical atrophy nor lateral or third ventricular enlargement. We have observed that both medical and schizophrenic patients share significant enlargement in lateral ventricular size in comparison to normal controls. However, an abnormality unique to the schizophrenic patient is CT scan changes consistent with prefrontal, but not generalized, atrophy. Results from our completed MRI study provide additional support for the notion of structural brain changes in schizophrenic patients. Specifically, enlargement of the cerebral ventricular system is found.

4. In a completed double-blind controlled investigation, we have observed that the calcium channel blocker, verapamil, is without therapeutic effect in neuroleptic-free patients. Significant increases in levels of plasma in CSF HVA were, however, observed.

5. In a completed study of twelve patients, we have observed significant improvement in psychosis ratings when alprazolam is added to a stabilized regimen of neuroleptic treatment. These clinical effects appear to be divisible into responders (5 of 12 patients) and nonresponders (5 of 12 patients; two partial responders) categories. In comparison with nonresponders, responders have greater prefrontal atrophy as seen on CT scan and greater alprazolam-induced decreases in levels of plasma HVA than nonresponders. We have also observed that some neuroleptic-treated patients with high residual symptoms respond well to the addition of nalmefene, a long-acting opiate antagonist.

6. One of the most consistent findings from our PET studies is that treatment with the typical neuroleptic, fluphenazine, increases glucose utilization in subcortical structures, notably the caudate and putamen. The interpretation of this increase in glucose utilization is compatible with the notion that fluphenazine decreases net dopaminergic activity in these structures since dopamine is an inhibitory neurotransmitter. PET studies of clozapine treatment are currently underway to examine whether this atypical neuroleptic produces similar effects in these key structures which mediate extrapyramidal movement.

7. 2-DG produces marked metabolic stress in normal subjects and in schizophrenic patients. The response is characterized by increased levels of cortisol and ACTH. Although plasma levels of HVA were significantly increased in both patients and controls, schizophrenic patients showed a significantly greater 2-DG stimulated increase in plasma HVA, despite neuroleptic treatment, than did controls. These data suggests that the modulation of presynaptic dopamine

activity, which occurs with neuroleptic treatment, is not rigid but rather subject to modification by stress.

8. Our initial experience with clozapine supports the notion that some poor neuroleptic-responsive patients improve considerably when treated with clozapine.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Schizophrenia is a major public health problem in the United States. This project attempts to study possible etiologic factors in schizophrenia, to develop a better understanding of mechanisms underlying current pharmacologic treatments and to improve the overall current pharmacotherapy of schizophrenia.

Our recent findings with regard to change in levels of plasma HVA during neuroleptic treatment and their demonstrated relationship to clinical response are important to the field as they suggest the possibility that monitoring levels of plasma HVA may be useful as a marker for the antipsychotic effects of neuroleptics. This work, focusing on change in dopamine system activity during neuroleptic treatment, is being pursued further using debrisoquin administration strategies to enhance the CNS HVA "signal." The indication that the antipsychotic process may involve a step-wise process, rather than a direct relationship with dopamine receptor blockade, provides new opportunity for strategies to augment neuroleptic response.

The introduction of clozapine into clinical practice will represent an important improvement in the pharmacotherapeutic armamentarium for schizophrenia. Our studies focusing on the clinical response and the underlying biological mechanisms by which clozapine produces its clinical effects holds significant importance to the field of psychiatry as well as for schizophrenia.

Our findings from CT studies are important as they add continued support for the notion of structural pathology in the frontal cortex of schizophrenic patients. Confirmation of ventricular enlargement by MRI is an important contribution to the field. Our inability to demonstrate the trait nature of the balance between positive and negative symptoms in schizophrenia and their lack of relationship to abnormality by CT scan raise into question the notion that structural brain abnormality is associated with specific types of schizophrenic symptoms.

Data from our follow-up study underscored the importance of symptoms in determining outcome among schizophrenic patients. Thus, agents which reduce symptomatology even modestly can result in meaningful improvement in functioning. This study may have significant economic implication for the treatment of patients with schizophrenia.

New treatment strategies which develop from this work would have considerable importance to the field of psychiatry and to the estimated 2 million patients suffering from schizophrenia in the United States. Our research ward is well-suited to studying the clinical and biological affects of pharmacotherapy of schizophrenia; we have had success in longitudinal studies of levels of plasma HVA and in the demonstration of the augmentation of neuroleptic antipsychotic effects by alprazolam.

PROPOSED COURSE

1. We will continue to study the implications of our plasma HVA data with regard to predictors of antipsychotic response in patients with schizophrenia. Using the debrisoquin technique and comparisons of plasma and CSF levels of HVA, we hope to develop a more clear understanding of neuroleptic/dopamine system interactions.
2. We will continue to pursue studies of clozapine and other strategies for augmenting typical neuroleptic response.
3. The analysis of data from our follow-up study will help to orient our thinking about factors relating to the course of schizophrenia. This study has already proven the feasibility of follow up studies at the NIMH and suggests the need for continued longitudinal study.
4. Collaboration with the NIMH PET group has increased over the past year and holds promise for developing new insights into not only the pathophysiology of schizophrenia but also the mechanism of action of neuroleptic drugs.
5. We will apply the developing strategies of eye tracking and neuroimmunologic assessment to outpatient and inpatient studies of schizophrenia. During the coming year we hope to be able to better assess how these variables may provide additional predictive or pathophysiologic information regarding schizophrenia.

PUBLICATIONS

Breier A, Arora PK, Pickar D, Wolkowitz OM, Paul SM. Metabolic stress produces rapid immunosuppression in humans. Arch Gen Psychiatry 1987;44(12):1108-9.

Breier A, Wolkowitz OM, Doran AR, Roy A, Boronow J, Hommer DW, Pickar D. Neuroleptic responsivity of negative and positive symptoms in schizophrenia. Am J Psychiatry 1987;144(12):1549-55.

Doran AR, Boronow J, Weinberger DR, Wolkowitz OM, Breier A, Pickar D. Structural brain pathology in schizophrenia revisited: prefrontal cortex pathology is inversely correlated with CSF levels of homovanillic acid. Neuropsychopharmacology 1987;1(1):25-32.

Pickar D. Recent perspectives on the mechanism of action of neuroleptic drugs: implications for a time-dependent model. Schizophr Bull 1988;14(2):255-68.

Pickar D, Breier A, Kelsoe J. Plasma homovanillic acid as an index of central dopaminergic activity: studies in schizophrenic patients. NY Acad Sci (in press).

Pickar D, Breier A, Wolkowitz OM, Doran AR. The biochemical basis for the antipsychotic effects of neuroleptics. In: Cazzullo GL, Invernizzi G, Sacchetti E, Vita A (eds), Etiopathogenic Hypotheses of Schizophrenia: The Impact of Epidemiological, Biochemical and Neuromorphological Studies. London, MPT Press 1987, pp. 175-80.

Pickar D, Breier A, Wolkowitz OM, Pato C. Profiles of the pharmacologic response of positive and negative symptoms in schizophrenia. Psychiatrie and Psychobiologie 1987;2(4):277-87.

Shelton RC, Doran AR, Pickar D, Weinberger DR. Cerebral structural pathology in schizophrenia: evidence for a selective prefrontal cortical defect. Am J Psychiatry 1988;145(2):154-63.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02184-06 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Depression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Pickar, Chief, Section on Clinical Studies NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

PROJECT HAS BEEN DISCONTINUED



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02187-05 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Diazepam Infusions as a Measure of Benzodiazepine Receptor Sensitivity in Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D.W. Hommer, Staff Psychiatrist, NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

PRINCIPAL INVESTIGATOR HAS LEFT INSTITUTE - PROJECT HAS BEEN DISCONTINUED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02345-01 NS
PERIOD COVERED October 1, 1987 to September 30, 1988		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) EYE MOVEMENTS IN PSYCHIATRIC AND NEUROLOGIC PATIENTS AND NORMAL VOLUNTEERS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Robert E. Litman, Senior Staff Fellow, Section on Clinical Studies, NS, NIMH Others: D. Pickar Acting Chief NS, NIMH D.W. Hommer Medical Officer VA C.N. Pato Medical Officer DCR, NIMH T. Clem Biomedical Engineer BEIB, NIH		
COOPERATING UNITS (if any) Department of Psychiatry, VA Medical Center, Seattle, WA; Schizophrenia Research Branch, Division of Clinical Research, NIMH; Biomedical Engineering and Instrumentation Branch, NIH		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Clinical Studies		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.25	OTHER: 0.25
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Disorders of eye movement</u> , particularly <u>abnormal smooth pursuit eye movement</u> , remain one of the few specific neurologic findings in <u>schizophrenia</u> . Despite many studies supporting the presence of eye movement disorders in schizophrenia, their precise nature and how they relate to the etiology and treatment of schizophrenia remain unclear. This project attempts to better define the pathophysiology of <u>eye movement disorders</u> by studying patients with schizophrenia, other <u>neurologic disorders</u> and normal controls utilizing a unique computerized <u>infra-red oculo-graphic recording</u> system which, in contrast to previous studies, allows precise quantitative measurement and analysis of eye movement. We have observed and quantified differences in smooth pursuit performance (<u>smooth pursuit gain</u>) and <u>saccadic intrusions</u> in smooth pursuit between schizophrenic patients and controls. We observed no effect of <u>neuroleptic medication</u> or clinical status on these differences in schizophrenic patients, confirming previous findings. However, in tests of <u>saccadic eye movement</u> , neuroleptic medication reduces the <u>amplitude</u> of <u>predictive saccades</u> . We observed in schizophrenic patients that the performance of smooth pursuit eye movement and the ability to make <u>anticipatory saccades</u> in <u>visually guided saccadic eye tracking tasks</u> is correlated with performance on the <u>Wisconsin Card Sort</u> , a test of <u>frontal lobe function</u> . We also observed that <u>caffeine</u> , a dopamine agonist, improves smooth pursuit performance by reducing saccadic intrusions in normal controls. Since a deficit in <u>dopaminergic neurons</u> arising from <u>frontal cortex</u> has been implicated as a possible mechanism for signs and symptoms in schizophrenia, our proposed course of study includes further comparison of eye movement with other tests of frontal lobe function and further study of the effects of dopamine agonists on eye movement in schizophrenic patients and controls. In addition, since it has been shown that deficits in eye movement may be inherited by unaffected <u>first degree relatives</u> of schizophrenic patients, we plan to study eye movement and other tests of frontal lobe function in family pedigrees in collaboration with the Unit on Molecular Neurogenetics.		

PROJECT DESCRIPTION

This project is part of the research program of the Section on Clinical Studies of the Clinical Neuroscience Branch. This Section conducts clinical research on the 4E Nursing Unit and the ACRF of the Clinical Center.

Although studies of eye movement in over ten separate laboratories have confirmed the existence of this abnormality in schizophrenia, the etiological pathophysiology of the eye tracking deficit in schizophrenic patients remains obscure. This stems in part from the fact that most workers studying eye movement in schizophrenia have used indirect, qualitative techniques to measure the overall quality of eye movement. Only two studies exist which directly measure eye position and eye velocity during eye movement tasks. An important facet of our project is our collaboration with the NIH Biomedical Engineering and Instrumentation Branch (Mr. Clem) which resulted in the development of an eye tracking system utilizing infra-red oculography which can exactly quantify and graphically display eye position over time via computer software program and computer hardware designed to convert infra-red voltage into digital information. Thus, we are able to derive exact numbers graphically displaying the following variables: eye position, velocity, average smooth pursuit velocity, maximum velocity of saccades, amplitude of saccades and saccadic reaction time.

We have performed eye tracking studies on normal controls and schizophrenic patients on and off neuroleptic and non-neuroleptic medication. We have measured the above variables in smooth pursuit, visually guided and predictive saccades and have correlated these with clinical status and neuropsychological measures of frontal lobe function. Other aspects of our research program include efforts to identify structural abnormalities in the brains of schizophrenics, especially in the frontal lobes, which may underlie eye movement abnormalities, by using CT and MRI techniques. As part of our outpatient program in schizophrenia, we have started to measure eye movement in schizophrenic follow-up patients in an effort to correlate eye tracking performance with outcome and other clinical measures which may predict course and outcome in schizophrenia.

Since eye tracking abnormalities are suggested to occur in unaffected first degree relatives of schizophrenic patients, we have been measuring eye movement in family members of schizophrenic patients and correlating eye movement measures with performance on the Wisconsin Card Sort, a neuropsychological test of frontal lobe function.

We have measured eye movement in response to an oral challenge dose of caffeine stimulant, a dopamine agonist, in normal volunteers.

We are also interested in characterizing eye movement abnormalities in other neurologic disorders including patients with HIV infection and brain lesions localized to the frontal lobe. A study to predict neurologic dysfunction utilizing eye tracking in neurologically impaired and unimpaired HIV infected patients is currently underway.

METHODOLOGY

Eye movements are recorded using an infra-red photoelectric limbus detection device (Eye Trac Model 210, Applied Science Laboratories, Waltham, MA). This

device has a response time constant of 4 msec, an accuracy level of better than 0.25 degrees of visual angle and a range of 15 degrees to the left and right of center. The subjects are seated 43 cm in front of a video monitor on which the target is displayed. The target is a small, bright square subtending a visual angle of less than 0.25 degrees. It is displayed against a dark background. The subject's head was secured by use of a bite bar. The infra-red source and photodiode detectors are positioned in front of each eye just beyond the eyelashes at the level of the lower lid. Only horizontal eye movements are recorded. The analog output of the infra-red eye tracking device samples at 1000 Hz using an analog to digital converter and processed data is stored for subsequent display and analysis using an IBM AT type computer. Before each run of data collection the system is electronically calibrated. This is done by asking the subject to look at targets in the center of the screen, 15 degrees to the left and 15 degrees to the right. When consistent values are obtained in each direction of gaze, calibration is complete and these values are used by the computer to calculate eye position and velocity from the stored data. Data is collected from both eyes, with best calibrated eye utilized for data collection.

Four different eye tasks are used. The first task is utilized to elicit smooth pursuit eye movement. In this task the target moves at a constant velocity of 16.67 degrees/sec, back and forth across the screen from 15 degrees to the right of center to 15 degrees to the left of center. The target moves for a total of 60 seconds, with a 15 second delay prior to and at the end of data collection, so that a total of 30 seconds of data is collected. The delay is to allow the subject to obtain optimal smooth pursuit eye movement.

The second eye movement task is designed to elicit visually guided saccades. The target steps back and forth 10 degrees to the right of center and 10 degrees to the left, at random time intervals. Ten cycles of eye movement are presented. A third eye movement task, also designed to elicit visually guided saccades, is presented but time intervals are constant and equal at 1 second each.

A fourth eye movement task is designed to elicit predictive saccades. In this task, unlike the two previous saccadic eye movement tasks, the target is not continuously present. The target appears at 10 degrees to the right of center. It remains on the screen for 0.75 seconds. It then disappears and the screen remains completely dark for 0.25 seconds. After this gap during which no target is present, the target reappears at 10 degrees left of center. This is repeated in the opposite direction to complete the cycle, and each test consists of ten such cycles. The disappearance of the target cues the subject to anticipate the appearance of the next target, and normal controls and most schizophrenic patients learn to anticipate target appearance in this task after one to four cycles.

Eye movement data is analyzed using an interactive graphics program designed in collaboration with the NIH Biomedical Engineering and Instrumentation Branch (T. Clem). The eye and target position are displayed as a plot of position versus time on a computer screen. The plot consists of 500 data points. Each data point represents the calculated mean eye position value over an interval of 5 msec, and 2.5 sec of eye data is displayed on the screen at a time. Instantaneous eye velocity and position as well as amplitude of any eye movement is measured directly from the screen via appropriate placement of the cursor.

Schizophrenic subjects tested are recruited from the 4E Nursing Unit and outpatient programs of the Section on Clinical Studies of the Clinical Neuroscience Branch. Schizophrenic subjects are rated on clinical scales which include the Bunney Hamburg Global Rating Scale, the Brief Psychiatric Scale, and the Scale for Assessment of Negative Symptoms. Schizophrenic subjects are oftentimes tested both on medication and when they are medication-free. Normal controls are recruited through the normal volunteer office. A diagnostic screening interview utilizing the Schedule for Affective Disorders and Schizophrenia is performed to rule out the presence of psychiatric and/or neurologic illness. All subjects undergo procedures as described. Each test takes approximately 1 minute, and subjects are allowed to close their eyes and rest between tests for several minutes.

MAJOR FINDINGS

1. Smooth pursuit gain (ratio of eye to target velocity), a measure of performance of the smooth pursuit movement task, is modestly but significantly decreased in schizophrenic subjects in comparison to normal subjects. This confirms the result of previous studies which have reported qualitative differences in the smooth pursuit tracking of schizophrenic patients. A more apparent difference concerns significantly increased numbers of saccadic intrusions during smooth pursuit and fixation, which most strongly differentiates schizophrenic subjects from normals. Smooth pursuit gain is correlated with performance on the Wisconsin Card Sort, a measure of frontal lobe function, in schizophrenic patients. Data analysis attempting to compare results of imaging studies with smooth pursuit eye tracking variables is currently in progress.
2. Neuroleptic medication does not improve or worsen smooth pursuit gain, nor does it influence the frequency of saccadic intrusions. Preliminary analysis of data indicates that neuroleptics cause hypometric saccades in predictive saccadic tasks, a phenomenon that occurs as well in Parkinson's Disease patients and is consistent with the effects of neuroleptics on the extrapyramidal motor system. Oral caffeine administration prior to eye tracking reduces the frequency of saccadic intrusions in smooth pursuit eye tracking in normal controls. These findings raise state/trait questions anew regarding the pharmacologic manipulation of eye movement.
3. Analysis of data for both saccadic and smooth pursuit eye movement in families of schizophrenic patients is currently in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Schizophrenia is a major public health problem in the United States. This project attempts to better define the functional neuroanatomy and neurophysiology of eye movement, and the eye movement disorders in schizophrenia, which may be related to location and the nature of the deficit(s) in schizophrenia. Clinically, correlation of eye movement variables with clinical measures, neuropsychological tests, imaging studies and treatment response may help to provide objective measures for use in the diagnosis and treatment of patients with schizophrenia. Correlation of smooth pursuit and saccadic eye movement performance with neuropsychological measures testing frontal lobe function has implication for the etiology and diagnosis of schizophrenia. Our findings of changes in smooth pursuit function after oral caffeine challenge but not after

chronic neuroleptic treatment may have implications in the pharmacotherapy of schizophrenia. Finally, eye movement disorders in families of schizophrenic patients may serve as genetic markers of the illness, and their study in conjunction with molecular genetic research of schizophrenic family pedigrees can provide further knowledge regarding the heritability of schizophrenia.

PROPOSED COURSE

1. We will continue to study eye movement in normal controls, schizophrenia and other disorders to better understand their anatomic and physiologic underpinnings. To do so we will develop new eye movement paradigms designed to more strongly elicit abnormal eye movement.
2. We will continue to investigate correlation between eye movement disorders in schizophrenia with other significant clinical and laboratory measures. We will attempt to correlate eye movement performance with outcome measures in our outpatient follow-up study. We will further investigate the heritability of eye movement by studying eye movement disorders in family members of schizophrenic patients and mono- and dizygotic twin pairs with schizophrenia.
3. We plan to further investigate the clinical use of dopamine agonist agents in schizophrenia and their effects on eye movement disorders, both after challenge dose as well as after chronic treatment.

PUBLICATIONS

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02346-01 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunology of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.H. Rapaport, Senior Staff Fellow, NS, DIRP, NIMH

Others: D. Pickar	Acting Chief	NS, NIMH
S. Paul	Chief, Sec on Mol Pharmacol	NS, NIMH
C. McAllister	Staff Fellow	NS, NIMH
C. Pert	Guest Researcher	NS, NIMH
J. Hill	Guest Researcher	NS, NIMH
M. Markwell	Guest Researcher	NS, NIMH
D. Brenneman	Head, Unit on Neurochemistry	LDN, NICHHD

COOPERATING UNITS (if any)

Section of Molecular Pharmacology, NSB, NIMH and Laboratory of Developmental Neurobiology, NICHHD

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1.75

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project studies the immunology of schizophrenia and encompasses three lines of investigation: studies of possible autoimmune and infectious etiologies of schizophrenia, of cerebral spinal fluid for antibodies, lymphocytes, or cytotoxin factors, and of the in vivo effects of antipsychotic agents on immune function. One major task of current research is the investigation of genotypic and phenotypic markers of peripheral lymphocytes. In particular, we are studying the HLA haplotypes of patients with schizophrenia and simultaneously evaluating markers of T and B lymphocyte subpopulations in selected patients using flow cytometry. Preliminary evidence indicates that approximately 30% of schizophrenic patients tested have an elevated number of CD5-positive B lymphocytes present in their peripheral blood. This finding is intriguing because approximately 30% of patients with rheumatoid arthritis and Sjogrens syndrome have similar elevations in these cells that spontaneously produce autoantibodies.

We are concurrently evaluating CSF samples for antibodies, lymphocytes, or cytotoxic or cytotropic factors in an attempt to investigate possible central nervous system evidence of immune or infectious contributions to the pathology of schizophrenia. These nascent studies involve autoradiography, immunohistochemistry, and in vitro measures of cell growth, and are being done in collaboration with Dr. Candice Pert and Peptide Designs.

The third line of investigation involves the use of flow cytometry and mitogen stimulation to evaluate the acute in vivo effects of typical and atypical antipsychotic agents on immune function. Lymphocytes from patients on the 4-East research ward are studied on a regular basis in order to test whether fluphenazine, thioridazine or clozapine modify the phenotype expression on function of lymphocytes from schizophrenic patients under drug free and medicated conditions.

PROJECT DESCRIPTION

This project is part of the research program of the Section on Clinical Studies of the Clinical Neuroscience Branch and is being conducted on the 4-East Nursing Unit and the ACRF of the Clinical Center.

Although there has been enormous clinical and research efforts to find etiologies for schizophrenia and investigation of the actions of antipsychotic aspects, there have been very few comprehensive studies of the role of the immune system in schizophrenia, and the effects of antipsychotic agents on immune function. We have recently initiated a series of systematic experiments evaluating the HLA system, phenotypic markers of T and B cells, lymphocyte function, antibody type and function; and other markers of immune and autoimmune function in order to look at this question. We are utilizing flow cytometry, mitogen stimulation, autoradiography, immunohistochemistry, measures of in vitro cell mortality, and immunology tests available through the clinical center in the aforementioned studies.

METHODOLOGY

We have begun to study lymphocyte phenotype by flow cytometry. This technique involves the using of 100 μ l of whole blood and staining of peripheral lymphocytes with fluorescently labeled monoclonal antibodies. The lymphocytes then pass through the flow cytometer, which uses a laser beam to determine the size, complexity, and fluorescent label present on the cells. This allows one to rapidly and accurately determine the phenotype and number of lymphocytes present.

A second technique employed in these studies is mitogen stimulation. Lymphocytes are separated from peripheral blood and then are cultured in the presence mitogenic stimulants PHA or Con.A it and tritiated thymidine. The rate of incorporation of tritium into the lymphoblasts gives a measure of lymphocyte function.

The autoradiography and immunohistochemistry studies use rat brain tissue slices and secondary monoclonal antibodies to study peripheral serum samples and CSF samples for autoantibodies.

The HLA study and our other investigators of autoimmune parameters employ the services of the HLA and clinical immunology laboratories.

MAJOR FINDINGS

We have found that approximately 30% of schizophrenic patients studied have increase numbers of CD5-positive B lymphocytes. This finding is similar to what is seen in rheumatoid arthritis and Sjogren's syndrome. Individuals with increased numbers of CD5 lymphocytes in these disorders spontaneously produce autoantibodies. Our finding is intriguing because it may indicate that an autoimmune process is involved in some aspect of the pathogenesis of schizophrenia.

Our preliminary data indicates that there may be a relationship between a certain HLA haplotype A2 B44 DR6 DRW52 DQ1 and increased markers of CD5 positive B

lymphocytes. This finding may be important because it may help clarify the links between the control of CD5 positive B lymphocytes and known factors involved in immune regulation and function - the HLA system.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE INSTITUTE

Schizophrenia is a major public health problem nationally and internationally, and currently little is known about its etiology or the ramifications of neuroleptic treatment on in vivo immune function. In this project we are beginning to study, in a systematic and comprehensive way, the possible role of the immune system and infectious aspects in schizophrenia. Our recent findings that certain patients with schizophrenia have elevated numbers of CD5 B lymphocytes is intriguing preliminary evidence that aberrant immunological processes may be involved in some aspect of schizophrenia. This finding may be useful as a clinical tool for subtyping schizophrenic patients and may have important ramifications in investigations of the etiology of this disorder. Our recent finding that there may be an HLA haplotype association between increased numbers of CD5 positive B lymphocytes and schizophrenia may give evidence for a genetic-immunological component to schizophrenia.

Our in vivo studies of the effects of typical and atypical antipsychotic agents and schizophrenia may lead to important knowledge about the effects of these agents on immune function.

PROPOSED COURSE

We will continue our current studies of phenotypic markers, genotype markers, and immune function. We hope to further clinically characterize these patients who have elevated numbers of CD5 B lymphocytes. We also plan to sort out the CD5 positive B lymphocytes, see if they produce autoantibodies, and if so, characterize them.

We will continue our current studies of the effects of antipsychotic agents on in vivo immune function.

We will continue our studies of CSF, looking for antibodies, lymphocytes, and cytotoxic factors.

PUBLICATIONS

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02177-06 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Functions of Neuropeptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.N. Crawley, Chief, Unit of Behavioral Neuropharmacology, NS, NIMH
Others: J. Mastropaolo Staff fellow NS, NIMH
S. DeMesquita Visiting Scientist NS, NIMH
W. Berrettini Staff Psychiatrist CNG, NIMH
B. Martin Visiting Scientist NS, NIMH
J. Hsiao PRAT Staff fellow NS, NIMH

COOPERATING UNITS (if any)

Section on Clinical Neurogenetics, Clinical Neurogenetics Branch

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A. Microinjection of the CCK antagonist, proglumide, in the nucleus accumbens of rats, partially blocked hyperlocomotion in a darkened Digiscan activity monitor. This finding is the first evidence that endogenous CCK contributes to mesolimbic functions.

B. Characterization of the ventral tegmental pharmacology of CCK found that CCK modulated DA, i.e. CCK had no effect alone, but potentiated DA-induced hypolocomotion, when microinjected into the VTA. This effect was mimicked by unsulfated CCK-8, and by CCK-4, and was not blocked by proglumide, suggesting mediation by a central-type CCK receptor subtype. A central-type CCK agonist mimicked this effect.

C. Galanin is a 29-amino acid peptide which coexists with acetylcholine in the septo-hippocampal pathway of the rat. Intraventricular or intrahippocampal microinjection of nanogram doses of galanin blocked the ability of acetylcholine to reverse memory deficits on a t-maze delayed alternation task in rats lesioned in the nucleus basalis of Meynert-medial septal area. An antagonist of this inhibitory peptide is being sought as a possible approach for the treatment of some cognitive dysfunctions associated with Alzheimers disease.

D. Microdialysis for neuropeptides is being developed using different plastics which allow greater passage of the large, highly charged peptides. Radioimmunoassays have detected measurable levels of cholecystokinin in the nucleus accumbens, and measurable levels of galanin in the ventral hippocampus, of anesthetized rats.

PROJECT DESCRIPTIONObjectives:

Behavioral analysis of the functions of neuropeptides, particularly the functional significance of peptides coexisting with classical transmitters in mammalian brain.

Methods Employed:

(SEE: 1987 Annual Report, Project Number Z01 MH 02177-05 NS, pp 483-488)

Aseptic stereotaxic implantation of indwelling cannulae into brain nuclei of rats. Microinjection of neurochemicals into discrete anatomical nuclei. Intra-peritoneal injection of drugs. Behavioral analysis of neurochemical effects, including locomotion, seizures, grooming, stereotyped motor behavior, and T-maze alternation task. Histological verification of cannulae placement and injection sites.

Major Findings:

A. Our ongoing studies of the behavioral actions of cholecystokinin in the brain have shown that CCK potentiates DA-induced hyperlocomotion in the nucleus accumbens and that CCK potentiates DA-induced hypolocomotion in the ventral tegmentum. The pharmacology of the actions of CCK suggest that a peripheral-type CCK receptor mediates its actions in the nucleus accumbens, while a central-type receptor mediates its actions in the ventral tegmentum. Equimolar concentrations of sulfated and unsulfated CCK were equally effective in potentiating DA-induced hypolocomotion in the ventral tegmentum, with CCK-4 also active at about ten times greater doses. A new central-type CCK agonist, synthesized by Dr. Bernard Roques, Universite Rene Descartes, Paris, mimicked the action of CCK in the VTA. The peripheral-type CCK receptor antagonist, proglumide, was ineffective at blocking CCK in the VTA. Two new CCK antagonists, CR-1409 and L-364,718, which are selective for the peripheral-type CCK receptor but also bind with lesser affinity at the central-type CCK receptor, partially blocked the behavioral effects of CCK in the VTA. Topographical analysis of sites within the VTA, and sites dorsal, rostral, caudal, and lateral to the VTA, found that DA induced hypolocomotion throughout the VTA but not outside the VTA, and that CCK potentiated DA-induced hypolocomotion in the medial and caudal VTA.

B. To investigate the role of endogenous cholecystokinin in mediating exploratory locomotor activity in the mesolimbic pathway, a dark-induced hyperlocomotion paradigm was developed. Using novel objects in a Digiscan arena placed in a dark room, rats hyperlocomote in a manner similar to our standard hyperlocomotion induced by dopamine microinjected into the medial posterior nucleus accumbens. Dark-induced hyperlocomotion may therefore represent a non-pharmacological method for activating the mesolimbic pathway, possibly by inducing a higher rate of VTA neuronal firing that is more likely to release endogenous CCK. We found that microinjection of a cholecystokinin receptor antagonist, proglumide, 10-20 μ g, into the medial posterior nucleus accumbens, significantly inhibited dark-induced hyperlocomotion, while have no effect on baseline locomotion in the light. These

data are the first evidence suggesting that endogenous CCK contributes to behaviorally relevant functions of the activated mesolimbic pathway.

C. Galanin is a 29 amino acid peptide discovered in 1983 by Tatemoto and Mutt in the gut and in the central nervous system of mammals. In the owl monkey and the rhesus monkey, galanin coexists with acetylcholine in the nucleus basalis of Meynert neurons projecting to the cerebral cortex, and in medial septal neurons projecting to the hippocampus. In the rat, galanin appears to coexist with acetylcholine in the septo-hippocampal pathway only. John Mastropaolo established the most robust animal model of Alzheimer's disease, five ibotenic acid lesions of the nucleus basalis of Meynert-medial septal area, and a t-maze delayed alternation task (Wenk and Olton, 1987) in our laboratory. We found an intraventricular dose of acetylcholine (7.5 or 10 μ g) which reversed the memory deficit in delayed alternation in the lesioned rats, having no effect in sham-treated control rats. Higher doses of ACH (20 μ g) caused motor seizures, supporting the notion that ACH was having a direct central effect during this short time period. The muscarinic antagonist, atropine, but not the nicotinic antagonist, mecamylamine, blocked this action of ACH, suggesting that a muscarinic receptor mediates this ACH effect. A dose of ACH, 1 μ g, micro-injected into the ventral hippocampus, also significantly reversed the performance deficit in the lesioned rats.

Galanin, 100ng-500ng, ivt, attenuated the action of ACH on delayed alternation. Galanin (200 ng) also blocked ACH when coinjected into the ventral hippocampus. When administered alone, galanin (100ng - 1 μ g) had no effect on delayed alternation in either the lesioned or the sham control rats. These data suggest that galanin may act as an inhibitory modulator of ACH in the septo-hippocampal pathway with respect to memory processes. This action of galanin was seen in the present paradigm only in rats with lesions of basal forebrain cholinergic neurons. As suggested by Melander and Hokfelt, the inhibitory actions of galanin on cholinergic function may be especially deleterious in Alzheimer's disease, where the small percentage of surviving cholinergic neurons may increase their firing rate and release more peptide, which then could severely inhibit the modulatory actions of released acetylcholine on cognitive functions. Our current goal is to develop an antagonist to galanin, by first identifying and then modifying the biologically active amino acid sequence within the parent 29 amino acids (collaboration with Dr. Brian Martin). Administration of a galanin antagonist would answer two questions: 1) does endogenous galanin play a role in memory processes; and 2) will a galanin antagonist enhance memory in lesioned and/or normal animals.

D. Microdialysis has not yet been successfully applied to the study of peptide release because of the low percentage recovery of peptides through the commercially available dialysis membranes. Possible reasons include 1) the larger size of peptides, e.g. 1142 molecular weight for cholecystokinin and 3211 molecular weight for galanin, as compared to 190 for dopamine; 2) many charged groups on peptides; 3) stickiness of peptides to glass, plastic, and metal tubing. We are attempting to develop the microdialysis technique for peptides, since this is the best technology to answer the question of whether endogenous peptides are released during physiological, pharmacological, and behavioral activation of brain pathways which contain the peptide. With John Hsiao, we are testing different plastics, with larger molecular weight limits, different

coatings with bovine serum albumin and polylysine, different collection and storage methods, and different perfusion flow rates. Radioimmunoassay for cholecystokinin, in collaboration with Dr. Marge Beinfeld, St. Louis University, and for galanin in collaboration with Dr. Wade Berrettini, NIMH, have shown less than 1% recovery to date. However, using Carnegie Medicin probes in the nucleus accumbens for CCK and in the ventral hippocampus for galanin, detectable quantities of both peptides have been collected in 20 μ l samples from the anesthetized rat. If these findings are not an artifact, then very high concentrations (10^{-6} M) of those peptides are found in these localized regions, which may be amenable to functional studies.

Proposed course:

A. New central-type CCK antagonists will be tested in the VTA as they become available from the several pharmaceutical companies working on this problem. Dark-induced hyperlocomotion will be used as a paradigm for microdialysis in the nucleus accumbens to determine whether endogenous dopamine and cholecystokinin are released from the mesolimbic pathway during presumed high levels of mesolimbic neuronal activation.

B. The smallest biologically active sequence of galanin will be determined from behavioral testing of fragments cleaved enzymatically from the parent 29 amino acids. This sequence will then be modified to create a galanin receptor antagonist. The t-maze delayed alternation task will be used by Dr. DeMesquita as a paradigm for microdialysis in the ventral hippocampus, to determine whether endogenous acetylcholine and galanin are released from the septo-hippocampal pathway during performance of a memory task.

Significance to Biomedical Research and to the Program of the Institute:

Behaviorally active antagonists of the central-type CCK receptor may provide the basis for clinical trials of a combination of a lowered dose of a DA antagonist plus a CCK antagonist, as an antipsychotic treatment with reduced risk for development of tardive dyskinesias. A galanin antagonist might eliminate the hypothesized negative feedback exerted by endogenous galanin on cholinergic transmission, in brain pathways mediating memory processes. A preclinically active galanin antagonist may provide the basis for a clinical trial to test whether a galanin antagonist could improve one of the most debilitating consequences of Alzheimer's disease, loss of cognitive functions.

Publications

Crawley JN. Modulation of mesolimbic dopaminergic behaviors by cholecystokinin. Ann NY Acad Sci (in press).

Crawley JN. Behavioral analysis of the functional significance of peptide-transmitter coexistences. In: Fuxe K, Agnati L, eds. Receptor-receptor interactions. Wenner-Gren Center International Symposium Series 1987;48:531-44.

- Crawley JN, Hommer DW, Skirboll LR. Cholecystokinin-dopamine interactions: electrophysiological and behavioral studies. Sixth International Catecholamine Symposium (in press).
- Kaltwasser MT, Crawley JN. Oxytocin and cholecystokinin induce grooming behavior in the ventral tegmentum of the rat, *Brain Research* 1987;426:1-7.
- Stivers JA, Kaltwasser MT, Hill PS, Hruby VJH, Crawley JN. Ventral tegmental oxytocin induces grooming. *Peptides* 1988;9(1):223-31.
- Stivers JA, Crawley JN. Substance P antagonists block carbachol-induced "boxing" behavior at a site of coexistence in the rat prefrontal cortex. *Peptides* 1988;9(1):117-21.
- Stivers JA, Skirboll LR, Long R, Crawley JN. Anatomical analysis of frontal cortex sites at which carbachol induces "boxing"-like seizures in the rat. *Pharmacol Biochem Behav* 1988;30:129-36.
- Khosla S, Crawley JN. Potency of L-364,718 as an antagonist of the behavioral effects of peripherally administered cholecystokinin. *Life Sci* 1987;42:153-9.
- Mastropaolo J, Crawley JN. Behavioral evidence for increased cholinergic receptor sensitivity after nucleus basalis magnocellularis lesions in the rat. *Eur J Pharmacol* 1988;153:301-4.
- Crawley JN. Neuronal cholecystokinin. *ISI Atlas of Science (Pharmacology)* 1988;2:84-90.
- Moody TW, Merali Z, Crawley JN. Neural mechanisms and biological significance of grooming behavior. *Ann NY Acad Sci* 1988;525:281-90.
- Crawley JN. Attenuation of dark-induced hyperlocomotion by a cholecystokinin antagonist in the nucleus accumbens. *Brain Research*, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02178-05 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology of Anxiety

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.N. Crawley, Chief, Unit of Behavioral Neuropharmacology, NS, NIMH
Others: S. Cottingham PRAT staff fellow NS, NIMH
L. Morrow PRAT staff fellow NS, NIMH
S. Deutsch PRAT staff fellow NS, NIMH
A. Weizman Guest Researcher NS, NIMH
R. Weizman Visiting Scientist NS, NIMH
S. Paul Acting Chief, Sec Molecul Pharmacol NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Maudsley Reactive rat is a strain of Wistar inbred rat which shows "hyperemotionality", characterized by a high incidence of freezing and defecation in an open field. Dr. Cottingham is characterizing this strain behaviorally and biochemically. Maudsley Reactives bred in our vivarium showed the expected high levels of freezing and defecation in our open field videotracking apparatus, as compared to Maudsley Non-Reactive and Sprague-Dawley controls. However, the Reactives showed no differences on the learned helplessness syndrome, or on t-maze memory paradigms. No differences between MNR and MR strains were found in mRNA for TH in adrenal gland or locus coeruleus, in mRNA for GAD in cortex, hippocampus, cerebellum, or striatum, or in mRNA for the gamma subunit of the GABA-A receptor in cortex or hippocampus. The MR had a significantly suppressed immune function, as measured by markers for t-cell and β -cell proliferation.

OTHER PROFESSIONAL PERSONNEL:

E. Ginns	Chief, Unit on Mol Neurogenetics	NS, NIMH
B. Martin	Visiting Scientist	NS, NIMH
M. Rehavi	Guest Researcher	NS, NIMH
P. Montpied	Guest Researcher	NS, NIMH
C. McAllister	Staff Fellow	NS, NIMH
D. Pickar	Acting Chief	NS, NIMH

PROJECT DESCRIPTIONObjectives:

Analysis of central mechanisms mediating stress and anxiety. Use of genetic probes, measures of immune function, and activity of the benzodiazepine-GABA-chloride ionophore receptor complex for analysis of biochemical mechanisms or markers of stress responses.

Methods Employed:

(SEE: 1987 Annual Report, Project Number Z01 MH 02178-04 NS, pp 489-491)

Assays of 1) immunological markers, including t-cell and β -cell proliferation; 2) Northern blot and in situ hybridization for quantitation of mRNA for tyrosine hydroxylase, GAD, and GABA receptors; 3) in vivo binding of ^3H -Ro15-1788; 4) assay of muscimol-stimulated chloride flux; 5) behavioral paradigms including shuttlebox avoidance, learned helplessness, t-maze alternation, and open field exploration.

Major Findings:

Dr. Rob Drugan finished his postdoctoral fellowship in December, 1987, and accepted an assistant professor of Psychology position at Brown University. Dr. Cottingham began her PRAT fellowship in our laboratory in July, 1987, with the behavioral and biochemical analysis of the Maudsley Reactive (MR) rat, and its control strain, the Maudsley Non-Reactive (MNR) rat. As originally described, the MR rat showed freezing in an open field exploration task, using our computer assisted videotracking system, or a smaller plexiglas open field. The MR strain behaved normally on the learned helplessness task, and on t-maze memory tasks, but showed a small deficit on performance in the middle trials of a 25-trial shuttlebox avoidance task. Corticosterone levels appeared to be elevated in the MR only during the latter task. These results suggest that the MR strain is more sensitive to stress, but has no deficit in learning.

Assays for mRNA for TH in adrenal and brain, for GAD in cortex, hippocampus, cerebellum, and striatum, and for the gamma subunit for the GABA-A receptor in cortex and hippocampus, showed no significant differences between the MR and MNR strains. There was no difference between the strains on muscimol-stimulated chloride flux in cerebral cortex, or on ethanol potentiation of muscimol-stimulated chloride flux. There did appear to be a significantly lower ^3H -Ro15-1788 binding in several brain regions in the MNR as compared to the MR, possibly due to differential responses to the stress of the injection

procedure. Reproducible immunosuppression was seen in the MR as compared to the MNR, which to date is the most significant suggestion of a biological indicator of the innate hyperresponsiveness to stress in the MR strain.

Dr. Cottingham has applied the molecular biology techniques, used for characterization of Maudsley rats, to investigate stress in normal Sprague-Dawley inbred laboratory rats. Inescapable tailshock appeared to increase levels of mRNA for TH in the locus coeruleus. No differences were detected between rats who had equal levels of inescapable tailshock but different performance on the learned helplessness task, suggesting that mRNA for TH changes after a major physical stressor (shock), but is not sensitive to the psychological components of the learned helplessness paradigm.

Transfection of fibroblast cells with the gene for tyrosine hydroxylase has been accomplished by Drs. Cottingham, Rehavi, Ginns and Paul in our branch. These investigators are now testing the possibility that antisense mRNA for TH can enter the fibroblasts from the culture medium to block the activity of the transfected TH. The use of transfected genes, and of antisense to block endogenous genetic sequences, has significant therapeutic potential.

Proposed Course:

Studies of the Maudsley Reactive rat model for anxiety have not yet revealed the mechanism responsible for the "hyperemotionality" syndrome. Biochemical markers previously shown to be normal in Reactives and Nonreactives in basal conditions will be tested in those strains after stress paradigms. In addition, the subtraction technique for identification of unusual genetic components of the Maudsley rat may be attempted.

Tyrosine hydroxylase gene transfection studies will be attempted in vivo, to test whether the transplants of the fibroblast cells transfected with TH will produce TH, DOPA, DA, and NE in vivo, and reverse behavioral deficits, in rats and mice depleted of catecholamines by pharmacological lesions.

Significance to Biomedical Research and to the Program of the Institute:

Our studies of neural mechanisms mediating anxiety are designed to identify brain transmitters, receptors, effector systems, and genetic factors which may be responsible for syndromes of extreme and chronic anxiety. The molecular genetics approach, e.g. transplanting genes for enzymes or receptors deficient in syndromes such as Parkinson's disease, or e.g. blocking genes for synthesis of enzymes or receptors which may be excessive or aberrantly regulated in syndromes such as stress-related anxiety, is a novel therapeutic strategy for neuropsychiatric disorders.

PUBLICATIONS

Drugan RC, Deutsch SI, Weizman A, Weizman R, Vocci FJ, Crawley JN, Skolnick P, Paul SM. Molecular mechanisms of stress and anxiety: alterations in the benzodiazepine/GABA receptor complex. In: Weiner, Hellhammer, eds. Neural Control of Bodily Functions, in press.

Drugan RC, Morrow AL, Weizman R, Weizman A, Deutsch SI, Crawley JN, Paul SM. Stress-induced behavioral depression in the rat is associated with a decrease in GABA receptor-mediated chloride ion flux and brain benzodiazepine receptor occupancy, Brain Research, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02179-06 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Animal Models of Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.N. Crawley, Chief, Unit of Behavioral Neuropharmacology NS, NIMH
Others: S.M. Paul Acting Chief, Sect Mol Pharmacol NS, NIMH
D. Pickar Acting Chief NS, NIMH
P. DeWitte Visiting Scientist NS, NIMH
I. Mefford Senior Staff Fellow NS, NIMH
R. LaBarca Visiting Scientist NS, NIMH
S. DeMesquita Visiting Scientist NS, NIMH

COOPERATING UNITS (if any)

Clinical Brain Disorders Branch, NS, NIMH.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology, NS, NIMH

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unraduced type. Do not exceed the space provided.)

The microdialysis technique, for continuous in vivo sampling of extracellular fluid in anatomically specific brain sites, was established in our laboratory this year. Peripheral administration of haloperidol increased concentrations of DOPAC and HVA in the caudate nucleus, while peripheral administration of amphetamine decreased DOPAC and HVA in the nucleus accumbens as previously described, validating our methodology. Microinjection of nanomole quantities of cholecystokinin and of neurotension into the ventral tegmentum of rats was shown to increase DOPAC and HVA in the nucleus accumbens, for up to 2 hours. Intraventricular injection of antiserum from patients with Sydenham's Chorea produced a small, highly variable increase in DOPAC and HVA in the caudate nucleus of rats. Peripheral administration of alprazolam caused a potentiation of the effects of haloperidol on increasing DOPAC and HVA in the caudate nucleus and in the prefrontal cortex of the rat. Microinjection of apomorphine into the prefrontal cortex produced a significant decrease in DOPAC and HVA in the caudate nucleus of rats. Microdialysis in the awake, freely moving rat revealed a small increase in DOPAC and HVA in the nucleus accumbens during periods of hyperlocomotion in rats placed in a Digiscan activity monitor, as compared to periods of rest in a holding cage. The microdialysis technique appears to be useful for addressing many of the anatomical, biochemical, and behavioral hypotheses of dopaminergic mechanisms relevant to the etiology and treatment of schizophrenia.

OTHER PROFESSIONAL PERSONNEL:

G. Jaskiw Senior Staff Fellow
J. Hsiao PRAT Staff Fellow

CBD, NIMH
NS, NIMH

PROJECT DESCRIPTIONObjectives:

Application of animal models to study the biochemical basis of neuropsychiatric disorders including depression, schizophrenia, obesity, alcoholism, and Alzheimer's disease.

Methods Employed:

(SEE: 1987 Annual Report, Project Number Z01 MH 02179-05 NS, pp 493-495)

Behavioral testing for locomotion, feeding, memory, alcohol-induced chronic drug treatments, cannulation and microinjection into discrete anatomical loci. Microdialysis of extracellular fluid, followed by assays of neurochemicals from discrete anatomical loci, after drug treatments in anesthetized rats, and during behavioral events in awake, freely moving rats.

Major Findings:

A considerable portion of personnel time and budget expenditures for our Unit this year has been invested in establishing and adapting the microdialysis technique in our laboratory. Dr. Crawley learned about this powerful new methodology for *in vivo*, continuous sampling during foreign travel, when she visited the laboratory of Dr. Urban Ungerstedt in Stockholm, Sweden, before attending a Wenner-Gren Conference in Stockholm. Dr. Ungerstedt developed a miniaturized, standardized dialysis probe, a non-pulsing low speed pump, and a liquid switch between gas-tight syringes, which are now commercially manufactured by Carnegie-Medizin in Sweden and sold through Bioanalytical Systems in West Lafayette, Indiana. Assays for dopaminergic metabolites were learned from Dr. Ivan Mefford, employing standard high pressure liquid chromatographic techniques which are sensitive down to about 1 nanogram per 20 microliters of dialysate. Dr. Crawley became further educated by visiting another established microdialysis laboratory, that of Dr. Robert Schwarcz, Maryland Psychiatric Institute in Catonsville, Maryland, which provided a demonstration of microdialysis in awake rats. In addition, Dr. Crawley hosted seminars and all-day visits in our institute by Dr. Ungerstedt (microdialysis in anesthetized animals) and by Dr. G. DiChiara (University of Cagliari, Italy, expert in microdialysis for dopamine and metabolites in awake animals).

Several scientists have joined our efforts in microdialysis experiments to study dopaminergic functions. Dr. Philippe DeWitte, Associate Professor at the University of Louvain, Belgium, spent the summer of 1987 and November 1987 in our laboratory, studying the effects of neuropeptides microinjected into the ventral tegmental area on the release of dopamine in the nucleus accumbens. He found that both cholecystokinin and neurotension caused an increase in DOPAC and HVA in the nucleus accumbens of anesthetized rats. NT was more potent and its effects more long-lasting than CCK. Relatively high

concentrations of both peptides were necessary to increase DA metabolite levels. This finding suggests that the cell bodies of the mesolimbic pathway respond to high concentrations of peptidergic input, in terms of neuronal activation and release of transmitter at the terminal region. More physiological concentrations of CCK and NT do not appear to activate VTA neurons sufficiently to induce measurable changes in DA release. Previous neurophysiological studies, employing microiontophoresis of unknown concentrations of CCK and NT, may therefore have been detecting pharmacological, rather than physiological activation of VTA neurons.

Dr. Rodrigo LaBarca, University of Chile, had previously found that a benzodiazepine, alprazolam, potentiated haloperidol in increasing tissue concentrations of dopamine metabolites, in the prefrontal cortex but not in the caudate nucleus, using in vitro assays of post-mortem tissue in the rat. Alprazolam is an Upjohn compound being marketed as an anxiolytic-antidepressant. Dr. Pickar's group has evidence for an antipsychotic action of alprazolam given in conjunction with typical neuroleptics. Dr. LaBarca employed the microdialysis technique to measure in vivo, on line release of DA from prefrontal cortex and caudate nucleus of anesthetized rats after acute treatment with haloperidol, haloperidol + alprazolam, or vehicle. In all experiments, haloperidol increased DOPAC and HVA in both brain regions. In most experiments, alprazolam increased the response to haloperidol. In some experiments, alprazolam increased the response to haloperidol in the prefrontal cortex but not in the caudate nucleus. However, this apparent regional specificity was not confirmed by statistical analysis. These data, therefore, did not support the hypothesis that the putative antipsychotic actions of alprazolam act preferentially through the prefrontal cortex.

Dr. Rodrigo LaBarca also began a microdialysis study of the effects of antiserum from children with Sydenham's Chorea on dopamine pathways. The antibodies in the serum from these patients are being investigated by Drs. Mark Rapaport, Cathy McAllister, and Steve Paul, in our branch. It is suspected that the rheumatic fever virus may induce an antibody which serendipitously recognizes some amino acid sequence on the cell surface membrane of dopamine neurons in the brain, causing the dysfunctions of these neurons which lead to the development of Sydenham's Chorea. In some experiments, Dr. LaBarca found a small, (20-30%) transient increase in DOPAC and HVA in the caudate nucleus of rats intraventricularly administered 5-10 μ l of a 1:2 dilution of purified antiserum from a patient in Utah who suffers from Sydenham's Chorea. When Dr. LaBarca returned to Chile, Dr. Crawley continued this microdialysis project. Again, only some rats showed the small increase in DOPAC and HVA after antiserum administration. Varying the dilution, volume, and time course of injection did not amplify this signal. It was concluded that in vivo release studies on slices of caudate nucleus would be more appropriate than microdialysis for the preliminary screen of the effects of many different antisera on DA release.

Dr. George Jaskiw is dividing his research time between Dr. Daniel Weinberger's branch at NIMH, St. Elizabeth's Hospital, and microdialysis experiments in our laboratory. He and Dr. Weinberger are interested in the role of the prefrontal cortex in schizophrenia, in particular the relationship between dopamine terminals in the PFC and efferents from the PFC that mediate dopaminergic functions in the caudate nucleus and nucleus accumbens. Dr. Jaskiw is finding

that microinjections of apomorphine into the PFC, at doses which activate postsynaptic dopamine receptors in the PFC, decrease DOPAC and HVA levels in the caudate nucleus. This finding supports the concept that the mesocortical dopaminergic pathway influences neurons in the PFC which serve to inhibit release of DA in the caudate nucleus, presumably through a presynaptic receptor on the terminals of nigrostriatal neurons.

Dr. DeMesquita is an associate professor at Marshall University in West Virginia, who is an expert in the neuropharmacology of REM sleep. She is on sabbatical in our laboratory to learn microdialysis in awake and sleeping rats. She is funded through an NSF one-year career development award to spend this time in Dr. Crawley's laboratory. Dr. DeMesquita is working with Dr. Crawley in adapting the existing methodology for microdialysis in awake rats to our more complex behavioral testing equipment. We have succeeded in dialyzing 15-minute samples in the nucleus accumbens of hyperlocomoting rats in the Digiscan activity monitor test chamber. HPLC analysis showed a small increase in DOPAC and HVA in the nucleus accumbens during the period of locomotor activity, in our first pilot experiments.

Proposed Course:

The microdialysis technique will be further developed for our studies, including more flexibility and reliability of pump, probe, and connectors for paradigms in behaving rats, and a microbore HPLC assay for DA and for DOPAC and HVA in prefrontal cortex, where concentrations are 10-100 times lower than in caudate nucleus. The alprazolam experiment may be tried again in rats treated with alprazolam haloperidol chronically rather than acutely. Sydenham's antisera from other patients (Egyptian) may be tested as described for the first (Utah) patient's antiserum. Glutamate receptor subtype agonists and antagonists may be tested in the caudate nucleus to identify the transmitter in the PFC → caudate pathway mediating the inhibitory effect of PFC efferents on limbic DA release. We are most excited about studying the release of DA during behavioral events thought to activate dopaminergic pathways, e.g. hyperlocomotion (mesolimbic), stereotypy (nigrostriatal), stress responses (mesocortical). The release of neuropeptides which coexist with DA in these pathways is also being studied using microdialysis followed by radioimmunoassay (see Z01 MH 02177-03 NS).

Significance to Biomedical Research and to the Program of the Institute:

Our Unit is the only laboratory in NIMH with the mission of development of better animal models for neuropsychiatric disorders such as schizophrenia, anxiety, depression, obesity, alcoholism, and Alzheimer's disease. Our applications goal is to help develop rational, selective drugs for these illnesses. The microdialysis technique is expected to be a powerful tool for determining the functions of endogenous neurochemicals in animal models relevant to neuropsychiatric disorders.

PUBLICATIONS

Angel I, Stivers JA, Paul SM, Crawley JN. Site of action of anorectic drugs: glucoprivic- versus food deprivation-induced feeding. *Pharmacol Biochem Behav* 1987;27:291-7.

Suzdak PD, Glowa JR, Crawley JN, Skilnick P, Paul SM. Response to KT Britton et al. Sci 1988;239:649-50.

Suzdak PD, Paul SM, Crawley JN. Effects of Ro15-4513 and other benzodiazepine receptor inverse agonists on alcohol-induced intoxication in the rat. J Pharmacol Exp Ther 1988;245(3):880-6.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02180-06 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Electrophysiological Studies of Peptidergic and GABAergic Function in Mammalian Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D.W. Hommer, Staff Psychiatrist, NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Unit on Electrophysiology, Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

PRINCIPAL INVESTIGATOR HAS LEFT INSTITUTE - PROJECT HAS BEEN DISCONTINUED

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02182-06 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Toward the Visualization of Opiate Receptors in Living Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Pert

Guest Researcher

NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

PROJECT HAS BEEN DISCONTINUED



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02183-06 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Is Schizophrenia an Autoimmune Neuropeptide Receptor Disease?

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C.B. Pert, Guest Researcher, NS, NIMH

Others: P. Laing Visiting Associate & Guest Researcher II, NS, NIMH

M.A. Markwell Guest Researcher II, NS, NIMH

J.M. Hill Guest Researcher PD, NS, NIMH

B.M. Martin Visiting Scientist NS, NIMH

COOPERATING UNITS (if any)

Integra Institute; Peptide Design

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

4.0

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Diverse infectious agents such as bacteria and viruses have been implicated by epidemiologic studies as likely environmental factors which may either predispose, to or precipitate some cases of schizophrenia. For example, exposure to influenza in utero may increase the risk of developing schizophrenia later in life. Similarly, certain strains of influenza can directly precipitate a schizophreniform psychosis (e.g., the 1918 H1N1 pandemic strain). However, it is generally believed that influenza viruses do not infect the central nervous system in man, nor are they thought to reach the fetus, except in extremely rare circumstances. How can influenza predispose to or precipitate schizophrenia if it does not infect the brain itself? If schizophrenia is thought of not as a viral illness, but as an autoimmune disorder triggered by viruses, then these puzzling facts can be reconciled. Thus, autoantibodies induced by influenza may cross the placenta (as do antibodies to the acetylcholine receptor and the TSH-receptor) thereby causing subclinical brain damage which predisposes to schizophrenia. A similar mechanism could account for precipitation of schizophreniform psychosis by influenza. We have therefore tested twenty strains of influenza for their ability to induce anti-brain antibodies in rabbits, and find that certain H1N1 viruses induce autoantibodies to a 37kDa protein which is specific to brain where it is confined to gray matter. We have purified this protein to homogeneity and in collaboration with Dr. Brian Martin have obtained a partial amino acid sequence.

Project Description:Objectives:

To identify candidate autoantigens which may be the subjects of autoimmune reactions triggered by viruses which contribute to the pathogenesis of schizophrenia. To identify the putative pathogenic epitopes on viruses which initiate these reactions.

Methods Employed:

(SEE: 1987 Annual Report, Project Number Z01 MH 02183-05 NS, pp 505-507)

Western blot and immunoprecipitation techniques have been used to screen for the presence of antibodies to specific brain proteins in the sera of rabbits immunized with various human strains of influenza-A. Autoradiographic immunohistological techniques were used to analyze the regional distribution of autoantigens in brain. Fast-protein liquid chromatography was used in combination with SDS-electrophoresis to isolate candidate autoantigens.

Major Findings:

Rabbits immunized with H1N1 influenza-A viruses produced autoantibodies to a previously unknown brain-specific protein. The viruses used were human isolates and the protein is also present in human brain. These findings may therefore be relevant to human diseases. The protein was demonstrated by 2D-electrophoresis to be a minor component of human brain tissue, and autoradiographic studies showed that it was confined to gray matter. Partial amino-acid sequencing of the brain protein shows that it has significant homology with the hemagglutinin of influenza-A but not with other influenza proteins or with the hemagglutinins of other viruses. The region of the hemagglutinin implicated in this phenomenon is active in membrane fusion which tentatively suggests that the brain protein which it mimics may also have this property (e.g., at synapses).

Significance to Biomedical Research and Program of the Institute:

Schizophrenia is a crippling psychiatric disease which affects one percent of the general population. A complete, convincing understanding of its etiology would almost certainly lead to better therapeutic strategies and would place this psychiatric illness in a more "normal" context with other diseases of the body.

Proposed Course:

We now hope to clone and sequence the 37kDa brain antigen with a view to determining its function in the brain. This would also facilitate the development of immunoassays to determine whether patients with schizophrenia or other neuropsychiatric disorders produce antibodies to this protein. It is not inconceivable that a course such as this could ultimately result in the development of diagnostic tests for schizophrenia and Parkinson disease. However, it is first necessary to establish whether antibodies are produced to this protein in humans after infection with influenza or during the course of neuropsychiatric disorders.

Publications:

Pert CB, Knight JG, Laing P, Markwell MAK. Scenarios for a viral etiology of schizophrenia. Schizophr Bull 1988;14(2):243-7.

Laing P, Knight JG, Hill JMH, Harris AG, Oxford JS, Newman R, Webster RG, Markwell MAK, Paul SM, Pert CB. Influenza viruses induce autoantibodies to a brain-specific 37 kDa protein in rabbits. Proc Nat Acad Sci (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02189-05 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropeptides and their Receptors are Shared by the Brain and the Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C.B. Pert, Guest Researcher, NS, NIMH

Others: P. Sacerdote

Visiting Fellow

NS, NIMH

J.M. Hill

Senior Staff Fellow

NS, NIMH

M.R. Ruff

Guest Researcher

NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH Clinical Neuroscience Branch

SECTION Section on Brain Biochemistry

INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 4.0

PROFESSIONAL: 2.5

OTHER: 1.5

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neuropeptides have now been shown to regulate immune system function. Our work reveals that human monocytes have receptors and will respond chemotactically to numerous neuropeptides. Neuropeptides which we have reported on include β -endorphin and other opiates, substance P and bombesin. We have shown that the benzodiazepines are also potent chemoattractants. In this case we have directly demonstrated the presence of chemotactic receptors through ligand binding experiments. The presence of diverse, distinct neuropeptide chemotactic receptors on monocytes and other immune system cells suggests the existence of a neuroendocrine link between the brain and the immune system whose purpose is to integrate behavioral and emotional responses with immune system function.

In addition to the presence of neuropeptide receptors, we have also been able to demonstrate that human alveolar macrophages store and secrete the neuropeptide bombesin. Neuropeptide synthesis is, therefore, a general feature of various immune cell populations. Such results are consistent with a multi-directional communication network via neuropeptides and their receptors. The purpose of this network is to link the body's cellular defense and repair systems with the nervous and endocrine systems and thereby integrate the internal milieu of the whole organism. The flow of information in this network is perceived by the human organism's emotional and/or altered states of consciousness. Ultimately, this results in behavioral decisions at the whole organism level. Additional work has suggested that a major cause of human cancer, small cell lung carcinoma, may not, as previously thought, arise from lung epithelium but originates from hemopoietic cells when the normal macrophage mediated repair of lung tissue is deranged by continuous heavy smoking.

Project Description:

Objectives:

Neuropeptide effects on immune function: All considerations of health and well-being must necessarily attempt to understand immune system function within the context of the whole organism. For this reason, we have considered the role of a class of molecules termed neuropeptides - short chains of amino acids primarily known for their role in nervous system function. What is becoming abundantly clear, however, is that these compounds, far from acting solely within the confines of the brain, are ubiquitous and have pleiotropic effects, functioning as transmitters, growth hormones, and signal agents for all the body systems. We feel that the neuropeptides are key components of a network whose purpose is to integrate behavior and brain function with immunological and endocrine systems.

In order to establish these points, we have decided initially to examine the effects of neuropeptides in several ongoing model systems which deal with the immunological aspects of inflammatory responses, specifically the macrophage/granulocyte component. Macrophages figure prominently in many inflammatory processes, such as arthritis or gingivitis. Inflammation is known to have a neurogenic component, and neuropeptides (such as substance P), released from local nerves, appears to play a role in some inflammatory vascular reactions, as well as in arthritis. We questioned whether such locally released neuropeptides could exert some of their effects through immune cells such as the macrophage.

Our first objective was, therefore, to demonstrate a direct role for select neuropeptides in immune function. Additionally, we wished to consider the possibility that neuropeptides, as a general class of compounds, might have effects on immune function. Sporadic reports of neuropeptide effects on in vitro mast cell and lymphocyte function have been made over the last 10 years, however, no unifying concepts from this diverse (sparse) literature have emerged.

Small cell lung cancer as a disease of macrophages: Lung cancer is the leading cause of cancer death in the United States, and greater than 25,000 people die each year of a subtype of lung cancer known as small cell (oat cell) lung cancer (SCLC). We have recently proposed that SCLC arises not from lung epithelium, but rather from the macrophages present in the lungs of chronic smokers. Our interpretation of the etiology of SCLC emphasizes lung emphysema, inflammation, and tissue damage as a stimulus for myelopoiesis and recruitment of bone marrow derived macrophages into diseased lung. These cells then become transformed and give rise to the disease known as SCLC.

Our program is directed toward exploring similarities between SCLC cells and macrophages with the intent to develop novel therapeutic modalities. These studies will also focus on new aspects of inflammatory cell biology, particularly the conditions which may lead to cell transformation and progression towards overt neoplastic disease.

Methods Employed:

(SEE: 1987 Annual Report, Project Number Z01 MH 02189-04 NS, pp 509-516)

Major Findings:

The opiate peptides (e.g., enkephalin, β -endorphin) are potent chemoattractants for human monocytes. Pharmacologic specificity can be demonstrated through the use of various agonists and antagonists of the opiate receptor. These compounds are exceeding potent; activity can be detected at 10^{-14} concentrations. Several other neuropeptides have been found to have chemotactic activity for human monocytes with similarly low active concentrations. These include the hypotensive, bradykinin like peptide substance P, and the hypothalamic peptide bombesin. Pharmacologic studies using closely related analogs of these peptides indicate that chemotactic effects are mediated by specific neuropeptide receptors.

The benzodiazepine (e.g., valium) class of drugs are also potent chemoattractants for human monocytes. Binding studies confirm the presence of receptors with the appropriate structure/function relationship. Benzodiazepines are among the most widely prescribed drugs in the USA, and no effects on macrophages or immune function have previously been described. The endogenous ligand for this receptor is a neuropeptide, only recently identified.

In addition to studies which have revealed a role for neuropeptides in monocyte chemotaxis, we have also been able to show that other cells also express highly specific chemotactic receptors for these compounds. Thus, tumor cells, which may also express migratory potential, will chemotax in response to selected neuropeptides. This response is not characteristic of all tumor cells but shows selectivity, both with respect to cells which respond and to peptides which are active. We have reported on the ability of neuropeptides such as bombesin, β -endorphin, substance P, and other to promote SCLC chemotaxis. Ongoing studies reveal that other highly metastatic tumors (e.g., breast) will also respond chemotactically to select neuropeptides. We have also shown that human spermatozoa will migrate chemotactically to various peptides. This new methodology, modified from our monocyte techniques, can now be utilized by researchers in human fertility who have, to date, not been able to assess sperm motility in any sensitive, quantitative fashion.

Small cell lung cancer: The cell of origin for SCLC has been speculative for 50 years but has focused on a neuropeptide secreting cell sparsely distributed in lung epithelium. Primarily, this is because SCLC cells synthesize a number of neuropeptides; most consistently bombesin. Our recognition of the importance of macrophages in inflammatory diseases and our studies on neuropeptide synthesis by macrophages prompted a direct test of the hypothesis that the putative neuropeptide secreting precursor was not a lung epithelial cell but another cell which figured prominently in the pathology of smokers lung, the macrophage. We were able to demonstrate four surface antigens, found only on macrophages and their precursors, to be present on cell lines and tumors of SCLC. These results are now confirmed and extended by other groups and we have interpreted these results to support our suggestion of a macrophage derivation for SCLC.

With regard to our studies on lung cancer, it should be noted that no effective therapy exists for this disease, and it is rapidly lethal; average life expectancy for untreated SCLC is 5-8 weeks. Oral cancer, although less common than lung cancer, is also associated with the use of tobacco products, and the etiology we propose for lung cancer is quite relevant to this disease as well. Our results have suggested a novel interpretation of the etiology of SCLC which suggests a number of unexplored therapeutic strategies.

The cell of origin for SCLC has been speculative for 50 years, but has focused on a neuropeptide secreting cell sparsely distributed in lung epithelium. Primarily, this is because SCLC cells synthesize a number of neuropeptides; most consistently bombesin. Our recognition of the importance of macrophages in inflammatory diseases and our studies on neuropeptide synthesis by macrophages prompted a direct test of the hypothesis that the putative neuropeptide secreting precursor was not a lung epithelial cell, but another cell which figured prominently in the pathology of smokers lung, the macrophage. We were able to demonstrate four surface antigens, found only on macrophages and their precursors, to be present on cell lines and tumors of SCLC. These results are now confirmed and extended by other groups and we have interpreted these results to support our suggestion of a macrophage derivation for SCLC.

Among the growth regulating hormones which control monocyte growth and differentiation are the interferons and colony stimulating factors. These and other immune hormones can now be evaluated systematically within this new conceptual framework for their effect on SCLC cells. The recent cloning of both of these hormones makes this an attractive approach as ample precedent documents the ability of these agents to modify leukemia cell growth in some settings. Various combined modalities may ultimately prove more efficacious than current treatments; limited to chemotherapy.

Significance to Biomedical Research and Program of the Institute:

We have identified a group of very potent compounds, the neuropeptides, which exert hormone effects on human monocytes and other cells. Our results, primarily utilizing a chemotactic assay system are novel and indicative of a broader role for these class of compounds in immune system function.

Neuropeptides are known to cause both mood and behavioral alterations when acting within the brain, and to be released into the body during various emotional and physical states. Because these same peptides have very potent effects on macrophages, as well as other components of the immune system, we feel that these compounds are a major class of biochemicals which subserve information exchange between the brain and the body. The functional interaction of the body's cells through networks of neuropeptides and their receptors would be expected to be critical to the health of the organism as a whole and suggests a mechanism by which emotional states can significantly alter the course and outcome of biological illnesses previously considered to be strictly in the somatic realm.

The physiological correlate of *in vitro* monocyte chemotaxis to these chemicals is still obscure but it seems likely that the local release of neuropeptides may have important effects on cell distribution and activities. Thus, to cite one example, the peptide substance P has been implicated in the vascular

erythematous reactions associated with inflammation and this peptide has very recently been shown to exacerbate experimental arthritis. Monocytes and lymphocytes (which also have substance P receptors) are prominent, locally present, cells which are primarily responsible for the degenerative changes which characterize arthritic lesions. Thus, it seems likely that this neuropeptide, by virtue of its ability to localise and activate immune cells, may have an important causative role in this and other inflammatory processes. The recent demonstration that depletion of substance P from the local area surrounding an arthritic joint resulted in a substantial amelioration of the disease suggests the feasibility and importance of a program directed toward the understanding of neuropeptide effects on macrophage and immune function.

The ability of neuropeptides to effect monocyte and some tumor cell migration suggests a further role for these agents in histogenesis and tissue organization, serving to recruit and/or maintain resident macrophage and other cell populations. Disseminated neoplastic diseases may, to some extent, develop as a result of neuropeptide regulated cell trafficking. Thus, tumor cells, which have detached from the primary mass, may respond to organ (site) specific neuropeptide attractants. An understanding of this process could be relevant to controlling tumor spread and may help explain the frequent metastasis of some tumors (e.g., SCLC, breast) to neuropeptide-rich body sites.

Proposed Course:

Explore clinical settings in which neuropeptide macrophage mediated responses may have significant causative or diagnostic potential. Various systemic diseases with an underlying neuropeptidergic component may be selected in altered macrophage neuropeptide chemotactic responses. An initial survey is being made of illnesses in which macrophages have a role, such as lung cancer to detect such alterations. Other diseases or conditions in which neuropeptides are known to play a role may also reveal alterations at the level of altered macrophage neuropeptide responsiveness. Neuropeptide therapy may prove useful in select illnesses by virtue of effects on macrophage or immune function. In vitro systems, such as chemotaxis, could be used to facilitate design of new drugs.

Establish the in vivo role for neuropeptides in macrophage function. Neuropeptides can be stimulated to be released at various sites (e.g., electrically, mechanically, chemically) and the accumulation of immune cells studied. These studies would support in vitro observations and could suggest the context in which physiological responses may occur. Such information could be important in understanding certain pathological states where macrophages accumulate and in devising ways to regulate their function.

Extended studies revealing antigenic similarities between macrophages and SCLC cells to explore functional similarities. This work will be directed at developing strategies for inducing cell differentiation and growth cessation with the aim of developing new therapeutic strategies. These studies will focus on possible lymphokine and monokine regulation of tumor growth/differentiation. We will also attempt to define the conditions which may result in transformation of inflammatory macrophages into cancer.

Biochemical studies aimed at characterizing neuropeptide receptors on monocytes and other immune cells. We will focus on receptor identification through

binding and cross-linking studies with the aim of purification and raising antibodies. These studies will make it possible to examine the mechanisms of receptor function leading to cellular activation. Anti-receptor antibodies with agonist or antagonist activity could be used experimentally and possibly therapeutically.

Publications:

1. Wiedermann, C.J., Sertl, K. and Pert, C.B. Neuropeptides and the immune system: substance P receptors in bronchus-associated lymphoid tissue of rat. Ann NY Acad of Sci, 496:205-210, 1988.
2. Sacerdote, P., Ruff, M.R. and Pert, C.B. Cholecystokinin and the immune system: receptor-mediated chemotaxis of human and rat monocytes. Peptides, 9:29-34, 1988.
3. Wiedermann, C.J., Sertl, K., Zipser, B., Hill, J.M. and Pert, C.B. Vasoactive intestinal peptide receptors in rat spleen and brain: a shared communication network. Peptides, 9:21-28, 1988.
4. Hill, J.M., Lesniak, M.A. and Pert, C.B. Co-localization of IGF-II receptors, IL-1 receptors and Thy 1.1 in rat brain. Peptides, 9:91-96, 1988.
5. Ruff, M.R., Sacerdote, P., Wiedermann, C.J. and Pert, C.B. Neuropeptide receptors are shared components of nervous and immune systems. In: Neuropeptides and Stress, Tache, Y. (Ed.), Springer-Verlag, (in press).
6. Wiedermann, C.J., Jelesoff, N.E., Pert, C.B. and Hill, J.M. Distribution of epidermal growth factor receptors in rat brain. Peptides, in (press).
7. Weber, R.J., Hill, J.M. and Pert, C.B. Regional distribution of Thy 1.1 in rat brain. J Neuroimmunol, 17:137-145, 1988.
8. Hill, J.M. Neuropeptides and their receptors as the biochemicals of emotions. In: Coping with Uncertainty: Biological Behavioral and Developmental Perspectives, Palermo, D.S. (Ed.), Lawrence Erlbaum Associates, Inc., New Jersey, in (press).
9. Hill, J.M. and Pert, C.B. Neurochemical basis of emotional behavior. In: Handbook of Neuropsychology, Balber, F. and Grafman, J. (Eds.), Elsevier, Amsterdam, in (press).
10. Hill, J.M., Lesniak, M.A., Kiess, W. and Nissley, S.P. Radioimmunohistochemical localization of type II IGF receptors in rat brain. Peptides, in (press).
11. Sacerdote, P., Wiedermann, C.J., Wahl, L.M., Pert, C.B. and Ruff, M.R. Visualization and characterization of cholecystokinin receptors on a subset of human monocytes and in rat spleen. Brain, Behavior & Immunity, in (press).

binding and cross-linking studies with the aim of purification and raising antibodies. These studies will make it possible to examine the mechanisms of receptor function leading to cellular activation. Anti-receptor antibodies with agonist on antagonist activity could be used experimentally and possibly therapeutically.

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7. Weber, R.J., Hill, J.M. and Pert, C.B. Regional distribution of Thy 1.1 in rat brain. J Neuroimmunol, 17:137-145, 1988.
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02190-05 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Distribution and Properties of Opiate and Other Brain Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C.B. Pert, Guest Researcher, NS, NIMH

Others: J.B. O'Neill

Guest Researcher

NS, NIMH

M.R. Ruff

Guest Researcher

NS, NIMH

C.M. Fraser

Pharmacologist

LNP, NINCDS

C.J. Venter

Chief, Recept. Biochem.

LNP, NINCDS

COOPERATING UNITS (if any)

Section on Receptor Biochemistry, Laboratory of Neurophysiology, NINCDS

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

2.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cross-linking covalently affixes reversible ligands to their recognition molecules for subsequent electrophoretic analysis. [¹²⁵I]-Tyr-²⁷-β-endorphin binds stereospecifically to rat brain membranes. The β-endorphin receptor, is perhaps a unique "epsilon" opiate receptor, but a larger body of evidence suggest that β-endorphin has a high affinity for most if not all of the opiate receptors types and subtypes. Cross-linking opiate receptors from different tissue sources can potentially reveal much information about the molecular basis of apparent opiate receptor heterogeneity. Cross-linking, however, only fixes 1% of the bound trace, and SDS-PAGE, while exquisitely sensitive, can fail to reveal substantial inter-molecular differences. Cross-linking was performed with the homo bi-functional reagent Disuccinimidyl Suberate (DSS). The iodinated cross-linking products of Tetrahymena, Leech CNS and rat brain membranes (both type 1 and type 2 conditions), appeared indistinguishable on SDS-PAGE gel with major cross-linking products at 58K and 100-110K. The strong cross-linked bands produced by incubation in the presence of the inactive opiate ((+)-naloxone) was completely abolished by the same (10⁻⁶M) concentration of its active isomer (-)-naloxone. Although we have thus far failed to distinguish between opiate receptors from a mammal, an invertebrate, and a unicellular organism, we continue to explore various conditions of binding, and electrophoresis, (e.g., reduced and unreduced) to examine possible receptor differences, both intra and inter species. Electrophoresis of proteolytic digests of cross-linked bands will be performed as a particularly sensitive method of distinguishing heterogeneity. Thus far, our cross-linking experiment suggest that the recognition molecule (the opiate receptor) which binds all opiate alkaloids and peptides is stable across evolution. As proposed, apparent physiological receptor heterogeneity is due to coupling to other membrane components.

Project Description:Objectives:

To map the neuroanatomical distribution of various chemically coded pathway in brain and to understand the neuroscientific significance of "multiple" receptors.

Methods Employed:

(SEE: 1987 Annual Report, Project Number Z01 MH 02190-04 NS, pp 517-519)

Major Findings:

One opiate delta receptor appears conformationally fixed, while the other appears capable of assuming mu, delta and kappa conformations.

We showed that β -endorphin labeled opiate receptor from rat, leech and *Tetrahymena* have the same molecular weights of 58 and 110Kd on SDS-PAGE. This suggests that the opiate receptor is stable across evolution.

Significance to Biomedical Research and Program of the Institute:

Pinpointing neurochemically coded tracts will enable us to determine the functional significance of each newly discovered pathway. The method can be used on human brain and ultimately should give information about the contribution of various neurochemically coded tracts to pathology.

Proposed Course:

We plan a sophisticated biochemical and immunological approach to further defining the molecular nature of opiate receptors. The type 1 opiate receptor complex with its advanced evolutionary accumulation in the forebrain of humans seems particularly worthy of further study (see Project Number Z01 MH 02182-03 NS, Toward the Visualization of Opiate Receptors in Living Human). We plan to study the brain distribution of insulin, transferrin, and their receptors to further demonstrate the breakdown in the distinction between "neuropeptides" and hormones.

Publications:

1. O'Neill, J.B., Pert, C.B., Ruff, M.R., Smith, C.C., Higgins, W.J. and Zipser, B. Identification and characterization of the opiate receptor in the ciliated protozoan, *Tetrahymena*, Brain Res. 450, 303-315, 1988

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02191-03 NS

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Receptors for the AIDS Virus and Other Neurotrophic Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C.B. Pert, Guest Researcher, NS, NIMH

Others: S. Craig M.A. Sc.

COOPERATING UNITS (if any)

LAB/BRANCH Clinical Neuroscience Branch

SECTION Section on Brain Biochemistry

INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5
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CHECK APPROPRIATE BOX(ES)

- | | | |
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| <input type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have demonstrated that the 60 kD protein previously characterized on a subset of T lymphocytes and named "T4", is another example of shared components between the brain and immune system. Thus, we have demonstrated that this cell surface molecule can be cross-linked to ¹²⁵I labeled AIDS virus envelope and immunoprecipitated by the Mab OKT4 in both T cells and brain. Our work shows that the neuropsychiatric effects of AIDS may not, as previously thought, be due to inflammatory processes but due to a direct neuronal infection of the virus. Gp120 was shown to be the most toxic neuronal substance known. Gp120, VIP and Peptide T all compete for binding to the Ty receptor. Gp120 apparently blocks VIP attachment. Apparently the hormone VIP needed for the maintenance of a healthy cell, is replaced by Peptide T which displaces Gp120. Peptide T and other homologs can block Gp120 killing effects on neuronal survival at 10-12m.

Peptide T and several rationally designed peptide analogs appear to bind with high affinity to the AIDS virus receptor, blocking viral infectivity at very low concentrations. We expect that synthetic peptide heteropolymers employing this core pentapeptide attachment sequence will provide valuable as an approach for a vaccine for AIDS.

This method and approach appears useful for exploring the presence of other virus receptors in the brain. For example, we have already observed that the Epstein-Barr virus which has been known to use the complement receptor on B cells as a receptor entry protein, may actually infect brain via the same receptor molecule which we have recently identified in brain.

Project Description:Objectives:

To find the cure for AIDS.

Methods Employed:

(SEE: 1987 Annual Report, Project Number Z01 MH 02191-02 NS, pp 521-524)

Major Findings:

By molecular characterization and immunoprecipitation, we have demonstrated that the AIDS virus receptor (T4) is present in human, monkey and rat brain in an indistinguishable form as that present on human T cells. We are isolating an endogenous peptide ligand that binds to these receptors, which will presumably mediate behavioral activity as well as immune function, from peptide extracts of rat brain. Meanwhile, synthetic peptides have been deduced with computer-assistance which bind with very high affinity (10^{-11} M) to AIDS virus receptors on rat brain membranes and displace radiolabeled viral envelope protein (gp120) at the same low concentrations. A series of peptide analogs have been constructed and a structure activity relationship for T4 receptors has been documented. This structure activity relationship appears to be constant whether human monocyte chemotaxis, AIDS virus infectivity, behavioral activity of rats after intraventricular injection, displacement of radiolabeled AIDS viral envelope, or inhibition of lymphocyte PHA mitogenesis or DR expression is studied.

Clearly, the AIDS virus receptor and its endogenous peptide ligand are yet another example of a neuropeptide receptor and ligand subserving intercellular communication throughout the brain and body.

Significance to Biomedical Research and Program of the Institute:

AIDS is the #1 public health problem in the USA. Unexpectedly, an understanding of neuropeptides and their receptors, a specialty of our Institutes's Program in general and my research specifically, is highly desirable to understand AIDS.

Proposed Course:

We will optimize peptide structure to prevent proteolysis and thus obtain "The Ultimate Peptide". We will use knowledge gained from this viral disorder to understand schizophrenia.

Publications:

1. Ruscetti, F.W., Farrar, W.L., Hill, J.M. and Pert, C.B. Visualization of a differentiation antigen of human helper T lymphocytes in primate brain. Peptides, 9:97-104, 1988.

2. Sacerdote, P., Ruff, M.R. and Pert, C.B. Vasoactive intestinal peptide: a ligand for the CD 4 (T4)/human immunodeficiency virus receptor present on brain and immune cells. J. Neurosci. Res., 18:102-107, 1987.
3. Hill, J.M., Ruff, M.R., Lesniak, M.A., Roth, J. and Pert, C.B. Molecular components common to the immune system and neurons: growth factors and their receptors. In: Bridge, T.P., Mirsky, A.F. and Goodwin F.K., eds. Advances in biochemical psychopharmacology: physiological, neuropsychiatric, and substance abuse aspects of AIDS, New York: Raven Press, 44:21-33, 1988.
4. Pert, C.B., Ruff, M.R., Ruscetti, F., Farrar, W.L. and Hill, J.M. HIV receptor in brain and deduced peptides that block viral infectivity. In: Bridge, T.P., Mirsky, A.F. and Goodwin, F.K., eds. Advances in biochemical psychopharmacology: physiological, neuropsychiatric, and substance abuse aspects of AIDS, New York: Raven Press, 44:73-83, 1988.
5. Pert, C.B., Smith, C.C., Ruff, M.R. and Hill, J.M. AIDS and its dementia as a neuropeptide disorder: role of VIP receptor blockade by human immunodeficiency virus (HIV) envelope. Annals of Neurology, Supp. to 23: s71-s73, 1988.
6. Farrar, W.L., Ruff, M.R., Hill, J.M. and Pert, C.B. Characterization of IL-1 receptor in brain. In: Bridge, T.P., Mirsky, A.F. and Goodwin, F.K., eds. Advances in biochemical psychopharmacology: physiological, neuropsychiatric, and substance abuse aspects of AIDS, New York: Raven Press, 44:35-44, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02152-09 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Discipline and Parental Control in Families with Affective Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Kochanska Research Psychologist LDP NIMH

OTHERS: L. Kuczynski Associate Professor Univ. of Guelph
M. Radke-Yarrow Chief LDP NIMH

COOPERATING UNITS (if any)

University of Guelph
Guelph, Ontario, Canada

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.40

PROFESSIONAL:

.20

OTHER:

.20

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mothers' discipline and control practices directed to their young children were studied in well and clinically depressed mothers. Depressive symptomatology has been linked to inappropriate control practices, but specific difficulties of depressed mothers and possible implications for their children's development have not been identified. Assessments of mother and child behavior are based on observations of their interactions in a naturalistic setting (see Annual Report MH 02144). This year analyses are a continuation of the previous year's work. The focus is on the specific control strategies used by well and depressed mothers. Analyses revealed that depressed mothers, compared to well mothers, have specific difficulties controlling their children. They show a pattern of verbal control strategies, indicative of less assertiveness and directness, and more fear of confrontation toward their children than the pattern manifested by well mothers.

Project Description

The determinants, contents, and effects of parental discipline and control practices in families with normal and clinically depressed mothers are investigated. Effective control becomes of particular importance in the second and third year of life when two conflicting developments occur: the child becomes able to regulate his/her own behavior and to comply with parental demands, but also becomes more overtly resistant toward caregivers, which is a manifestation of emerging autonomy (see Annual Report MH 02144).

If the parent is not able to maintain effective control of the child, and at the same time to promote the child's emerging autonomy, this may be a source of future dysfunctions. Maternal depression has often been associated with general maladaptive patterns of control, such as hostility, punitiveness, and low involvement. In our previous work we identified also a general pattern of interaction between depressed mothers and their young children, characterized by fear of confrontation and diminished ability to reach compromise (see Annual Report MH 02144). The focus now is on the specific strategies used by well and depressed mothers during their control interventions.

Methods

Control interventions of 33 well and 37 unipolar depressed mothers and their children (1 1/2 to 3 1/2 years of age) were analyzed. The basic paradigm is described in Annual Report # MH-02144. Each control episode occurring during 90 minutes of naturalistic interactions was coded starting with the mother's attempt to regulate child behavior and continuing until the issue was resolved or dropped. Details of the coding were described in previous annual reports. This year the analyses were focused on the verbal control strategies used by the mothers. We distinguished direct commands, indirect commands, unclear commands and hints, explanations, bargains, reprimands, and positive reinforcements.

Findings

The analyses revealed that depressed mothers showed a pattern of verbal control strategies indicative of diminished directness and assertiveness. They used fewer direct commands and reprimands, and more explanations than the nondepressed women. This pattern is suggestive of the avoidance of confrontation, and consistent with our previous findings regarding general interactive patterns of well and affectively ill mothers.

Proposed course.

Two manuscripts have been published in journals. A third one is being prepared. Future work will focus on the longitudinal aspects of control interactions between the mothers and their children.

Significance to Biomedical Research

Children of depressed parents are at greater risk for psychopathology and behavioral disorders than are children of normal parents. Research on child development has demonstrated that aberrant parental disciplinary practices are important contributors to children's disordered social and emotional development. How depression affects the parent's abilities to function in controlling child behavior is largely unresearched; yet this variable may contribute significantly in creating a pathogenic environment for young children.

Publications

Kochanska G, Kuczynski L, Radke-Yarrow M, Darby Welsh J. Resolutions of control episodes between well and affectively ill mothers and their young children. J Abnorm Child Psychol, 1987;15:(3)441-456.

Kuczynski L, Kochanska G, Radke-Yarrow M, Girnius-Brown O. A developmental interpretation of young children's noncompliance. Dev Psychol, 1987;23:(6) 799-806.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02156-09 I.DP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Personality of Children Reared by Normal and Depressed Mothers: Inhibited Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F. Bridges-Cline Guest Researcher LDP NIMH

OTHER: G. Kochanska Research Psychologist LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.05

PROFESSIONAL:

.50

OTHER:

.55

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study focuses on the role of early childhood inhibition in the development of pathological risk indicators in children of families with and without parental depression. Patterns of child behavior in the face of the unfamiliar (persons, places), such as behaviors expressing inhibited exploratory activity and social withdrawal, are observed at 2 to 3 years of age in semi-naturalistic but standard settings, which represent varied contexts of unfamiliarity. Analyses of these data revealed that six reliable dimensions of mother and child response styles could be empirically derived from our observation coding system. These dimensions meaningfully distinguish groups of children in our sample at this very young age. Comparisons across maternal diagnostic groups (nondepressed, major depressive, and bipolar) indicate that the single factor that distinguishes depressed and nondepressed mothers is labelled Anxious, Non-facilitative Involvement of Mother with Child, with both the major and bipolar mothers displaying higher levels of intrusive, awkward and negative behavior (e.g., critical of child's behavior) than the nondepressed mothers. Children of the bipolar mothers, as contrasted to those of the major and nondepressed mothers, typically show the highest levels of exploratory activity and confident, animated stranger approach and engagement. As a group the children of the major mothers typically show more cautious, less active exploration and stranger engagement than children in both the bipolar and nondepressed maternal groups. Children and mothers of the major depressive group are most notably distinguished from the bipolar group on the factor, Social Withdrawal and Flat Affect.

Project Description:

Reduced motor activity, anhedonia, disinterest in activity, and social withdrawal are behavioral characteristics that are often associated with depression. However, the role of early behavioral forms of these characteristics as precursors to later manifestations of depression has not been established. One of the early forms of these characteristics is behavioral inhibition, as revealed by the way in which the child engages with novelty or unfamiliarity. One purpose of this study is to identify and describe the patterns of response to unfamiliarity that are exhibited by young children of depressed and nondepressed mothers. Another question of interest is how mothers' handling of their children in these situations may differ. Of predictive interest is the consequence of these early behavioral patterns for later manifestations of disordered behavior.

Methods Employed and Major Findings:

Patterns of response to unfamiliarity are studied in children of depressed and nondepressed mothers at 2 to 3 years of age. The children's behavior has been videotaped in varied situations of unfamiliarity, such as entrance to an unfamiliar but attractive environment, and introduction to and interaction with an unfamiliar but friendly adult. Child behavior measures that are coded from these videotapes include: latency measures, such as latency to touch objects or toys in the entrance situation; proximity to and reliance upon mother in order to interact or explore; and levels of exploration and interaction such as scanning and looking, or retreating from stranger in contrast to actively manipulating objects or initiating interaction with the unfamiliar adult. In addition, the way in which the mother functions to facilitate or hinder the child's approach and engagement of the unfamiliar is studied.

Because of an interest in identifying patterns, not only of children's but also of mothers' response styles in these situations, both mother and child behavior measures were included in a factor analysis of these data. Six factors emerged from this analysis. Two of these factors are specific to the entrance situation: 1) Lack of Exploratory Activity, with Physical Proximity to Mother (a "child" response style), and 2) Mother-child Distance (a "mother-child" response style). Four of the factors are specific to the stranger situation: 1) Mother-Child Contact, with Child Withdrawal from Stranger (a "mother-child" response style), 2) Wary, Hesitant Approach of Stranger (a "child" response style), 3) Social Withdrawal and Flat Affect (a "child" response style), and 4) Anxious, Non-facilitative Involvement of Mother with Child (a "mother" response style). When the group distributions for these six factors are compared in terms of depressed (major and bipolar combined) versus nondepressed mothers, the single factor that distinguishes these two groups is the mother factor, labelled Anxious Non-facilitative Involvement of Mother with Child, with both the major and bipolar mothers displaying higher levels of intrusive, awkward, and negative behavior (e.g., being critical of the child's behaviors) than the nondepressed mothers. A more refined

Findings

picture emerges when the group distributions are contrasted by the different maternal diagnostic groups bipolar and major depressive. As a group, the children of the bipolar mothers, as contrasted to those of the major depressive and nondepressed mothers, typically show the highest levels of exploratory activity and confident, animated stranger approach and engagement, as reflected by the relative location of this group on the child factor scales of Wary, Hesitant Approach of Stranger, Social Withdrawal and Flat Affect, and Lack of Exploratory Activity with Physical Proximity to Mother. In contrast, as a group, the children of the major depressive mothers typically show more cautious, less active exploration and stranger engagement than children in both the bipolar and nondepressed maternal groups.

More complex differences between the bipolar and major depressive group are most dramatically evoked in the stranger situation. The two factors that most notably distinguish the major depressive from the bipolar maternal diagnostic groups are the child factor, labelled Social Withdrawal and Flat Affect, and the mother-child factor, labelled Mother-child Contact with Child Withdrawal from Stranger. Whereas the bipolar groups are extremely homogeneous and low on these factors, the major depressive group exhibits a far more scattered distribution that stretches toward the high end of the scales, with a number of extreme outliers located at the upper ends of the scales.

Significance to Biomedical Research:

Our observational coding system focusing on the behavioral inhibition of the 2 to 3-year-old children in our sample yielded a set of stable and interpretable response style patterns, which meaningfully distinguish groups of children at this very young age. Accomplishment of these objectives -- reliable assessment of the young child's behavioral expression of reduced activity and/or social withdrawal in the face of the unfamiliar -- is a significant step toward a better understanding of the developmental course of these characteristics and of their link to later manifestations of depression or other disordered behavior.

Proposed Course:

Further analyses are being conducted to examine the concurrent as well as predictive relations of these early response patterns with other child characteristics (e.g., sex, age, profiles of child affect, and areas of concern/behavioral problems) as reported by mothers and the child him/herself. A manuscript reporting the findings of these analyses will be prepared.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02164-08 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Changes and Psychological Functioning During Adolescence

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.J. Susman	Guest Researcher	LDP NIMH
OTHERS:	E.D. Nottelmann	Statistician	LDP NIMH
	G.E. Germain	Research Psychologist	LDP NIMH
	L.D. Dorn	Guest Researcher	LDP NIMH
	G.P. Chrousos	Senior Investigator	DEB NICHD
	G.B. Cutler	Senior Investigator	DEB NICHD
	D.L. Loriaux	Chief	DEB NICHD

COOPERATING UNITS (if any)

Developmental Endocrinology, NICHD

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.38

PROFESSIONAL:

.28

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The interrelations of psychological functioning and endocrine and physical growth factors in young adolescents are investigated. Participants are 56 boys and 52 girls, 9 to 14 years old, and their parents. Participants were evaluated three times, six months apart, on stage of pubertal development (Tanner criteria), hormone levels, and psychological status and behavioral functioning, including interactions with their parents. Relations between behavioral and hormonal changes in a potentially stressful situation (blood withdrawal and physical examination) and adjustment problems were examined. Parental support in relation to amount of distress behavior shown in the stressful situation was also examined. It was hypothesized that adolescents who showed high physiological reactivity (increase in cortisol level during the stressful procedure) would have more behavior problems and symptoms of anxiety and depression one year later than adolescents who showed no change or decrease in cortisol level. The hypothesis was partially supported. Adolescents who showed increases in cortisol level reported more non-aggressive behavior problems and more depressive symptoms one year later than the adolescents who showed no change or decrease in cortisol level. There were no differences among the groups on aggressive behavior problems or symptoms of anxiety. Also, parents showed more monitoring and regulating behavior toward adolescents who showed the most behavioral distress. Parental anxiety was positively related to adolescent cortisol level. Parents of boys showed distress to signs of behavioral distress in their child; parents of girls did not show this type of response.

Project Description

The purpose of the study was to examine relations between behavioral competencies and dysfunctions and endocrine and physical growth changes in young adolescents. The behavior of parents toward their adolescents also was examined.

Methods and Findings

The participants in the study were 56 boys and 52 girls, 9- to 14-year-old, and their parents. Participants were evaluated three times, six months apart, on psychological status, stage of pubertal development (Tanner criteria), and hormone levels. (Details of measurement are reported in previous annual reports of this project.)

The relation between behavioral and hormonal changes in a potentially stressful situation (blood withdrawal for assessment of hormone levels and a physical examination for pubertal staging) and adjustment problems was investigated. A second focus was on parental support in relation to amount of distress behavior exhibited by adolescents in the potentially stressful situation. There is evidence from studies of animals and humans that exaggerated behavioral and physiological (e.g., hormonal) reactivity to novel and challenging situations is related to problems of adjustment. The role of parent behavior is unclear.

Individual differences in physiological reactivity and behavioral reactivity were examined in terms of adolescents' short-term changes in cortisol level (changes across a 40-minute period) and adolescents' and parents' behavioral responses to the potentially stressful situation (blood withdrawal and physical examination). Cortisol level is a commonly used index of physiological reactivity and distress. Adolescent behavior was observed and coded for distress behaviors (e.g., complaints of pain, muscular rigidity, crying, and physical resistance). Parent behavior was observed for signs of anxiety as well as monitoring and regulating the adolescent's emotional and physical needs.

It was hypothesized that the adolescents who showed high physiological reactivity (increase in cortisol level across the 40-minute period), at the first time of testing, would have more behavior problems and symptoms of anxiety and depression, one year later, than adolescents who showed no change or decrease in cortisol level. The hypothesis was partially supported. Adolescents who showed increases in cortisol level reported more nonaggressive behavior problems and more depressive symptoms one year later than the adolescents who showed no change or decrease in cortisol level. There were no differences among the groups on aggressive behavior problems or symptoms of anxiety.

Parents showed more monitoring and regulating behavior toward adolescents who showed the most behavioral distress. Parental anxiety was positively related to adolescent cortisol level.

Significance to Biomedical Research

There is increasing emphasis on the importance of early detection of adolescents at risk for the development of behavior problems. The study of individual differences in adolescent and parental reactions in potentially stressful conditions may lead to early detection of patterns of behavior that predict later maladjustment.

Proposed Course

Manuscripts are in preparation and will be reported in MH-02231. This is a final report.

Publications

Susman EJ, Dorn LD, Fletcher JC. Reasoning about illness in ill and healthy children and adolescents: Cognitive and emotional developmental aspects. J Dev Beh Pediatr 1987;58:266-273.

Trickett PK, Susman EJ. Parental perceptions of childrearing practices in physically abusive and nonabusive families. Dev Psychol 24, in press.

Trickett PK, Susman EJ. Perceived similarities and disagreements about child-rearing practices in abusive and nonabusive families: intergenerational and concurrent family processes. In: Cicchetti D, Carlson V, eds. Theoretical perspectives and research on the consequences of child maltreatment. New York: Cambridge University Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02170-06 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychiatric Assessment of Infants and Toddlers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. Cytryn	Medical Officer (Psych.)	LDP NIMH
OTHERS:	T. Sherman	Research Psychologist	LDP NIMH
	D. McKnew, Jr.	Medical Officer (Psych.)	LDP NIMH
	A. Mayfield	Social Science Analyst	LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Affective Development

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.90

PROFESSIONAL:

1.40

OTHER:

.50

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to develop a nosology of psychopathology for children 2 to 4 years of age. Two observational instruments (see reports Z01 MH 02170-03,04, and 05) were developed that allow for reliable and systematic recording of the child's behavior. One instrument is used for a semi-structured play interview with a psychiatrist, when mother is not present. The second instrument is used to observe the child in interaction with mother. Children were rated on degree of risk for the later development of psychopathology based on their performance in the two settings. From a case by case analysis of the 16 children who were rated as being at high risk in both settings, a nosology for describing current worrisome behavior has been developed. The behavior was evaluated along four dimensions: quality of child's relationship with mother, child's dominant mood, child's ability to regulate his mood, child's mastery as revealed in play. This yielded four syndromes of deviant behavior. Syndrome D: the relationship to mother is detached, dominant mood is bland, mood expression is overcontrolled, and play is minimal. Syndrome S: the relationship to mother is physically clingy, yet emotionally isolated, the dominant mood is sadness, moods are poorly regulated, and play is absent. Syndrome A: the relationship to mother is angry, the dominant mood is anger, there is excessive expression of negative moods, and play is minimal. Syndrome SD: the relationship to mother is variable ranging from moments of warmth to excessive anger. The dominant mood is anger. When under the direct supervision of an adult the child can control the expression of his anger and engage in productive play. The children are structure dependent. When not under the direct supervision of an adult, the children lose control of their anger and their play becomes dysfunctional.

Project Description

The goal of this project is to develop a nosology of psychopathology for children two to four years of age. Two behavioral observation systems that allow for reliable and systematic recoding of the child's behavior are used. One instrument is used for a semi-structured play interview with a psychiatrist when mother is not present. The second is used for evaluation of the child's interaction with mother.

One hundred and twenty children, 2 to 4 years of age, (the sample is from the longitudinal study (Z01 MH 02155) were assessed using the two behavioral observation systems.

Methods and Major Findings

Each child's performance in each of the settings was evaluated independently. A rating (1 to 4) was assigned on degree of risk for the later development of psychopathology. Children who received ratings of high risk in both assessments (16 children) were examined in detail, and a nosology for describing their current worrisome behavior was developed. Their behavior was evaluated along four dimensions: relationship to mother, dominant mood, control of mood, and mastery as revealed in play. Each dimension of behavior was assessed in terms of child's facial expression, body posture, activity and absence of activity, and content and tone of child's speech. For each dimension, the modal or dominant patterns of deviant dysfunction were coded.

Rating the children on the four dimensions yielded four syndromes.

- 1.) Syndrome D - a.) Detached relationship to mother, b.) dominant mood is apathy, c.) mood expression overcontrolled, and d.) largely absence of play.
- 2.) Syndrome S - a.) Relationship to mother emotionally isolated, yet physically close, b.) dominant mood is sad, c.) both excessive expression of sadness and anxiety and lability of moods among the negative moods, and d.) absence of play.
- 3.) Syndrome A - a.) Relationship to mother is angry, which child best when left on his/her own in the presence of the mother, b.) dominant mood is anger, at times bordering on rage, c.) an excessive expression of anger and other negative moods, and d.) low frustration tolerance and low tolerance for adult intervention.
- 4.) Syndrome SD - a.) Relationship to mother is angry, but can improve when child is the focus of the mother's attention, b.) dominant mood is anger, c.) excessive expression of anger, and d.) impulsive, uncontrolled interaction with play materials, which can be improved when the child is under the direct supervision of an adult. (Structure dependent.)

Significance to Biomedical Research

The development of improved assessment instruments will help in understanding developmental patterns in very young children and will permit more sensitive evaluation of their strengths and vulnerabilities. This prospective information

not only adds to our understanding of the developmental course of affective illness, but may provide an informed basis for identifying children who are most at risk.

Proposed Course

Analyses are completed, and manuscripts are being prepared for publication in scientific journals.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH-02171-05 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protective and Risk Factors in Childrearing: Contributions of Fathers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. Richters Research Psychologist LDP NIMH

OTHERS: W.E. Wilson Research Psychologist DRG NIH

COOPERATING UNITS (if any)

Division of Research Grants, NIH

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.55

PROFESSIONAL:

.15

OTHER:

.40

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The husbands of depressed women may buffer their children from the stresses of living with a depressed mother by encouraging dialogue, maintaining stability and equilibrium within the family, and providing children with a supportive environment. But they may also exacerbate the stresses to which their children are exposed by not functioning effectively in these roles. The objective of the present study is to examine the functioning of spouses of depressed women.

Project Description:

The husbands of depressed women may buffer their children from the stresses of living with a depressed mother by encouraging dialogue, maintaining stability and equilibrium within the family, and providing children with a supportive environment. But they may also exacerbate the stresses to which their children are exposed by not functioning effectively in these roles. The objective of the present study is to examine the functioning of spouses of depressed women.

Method:

Data are from families participating in a follow-up phase of the longitudinal study (Z01 MH 02144), which includes parents with and without a history of affective disorder. Assessments of fathers include a psychiatric interview, an interview concerning the father's role in child rearing and family functioning, and intensive interviews with mothers concerning the marriage and family life. In addition, fathers were observed in the laboratory with their children and wives.

Findings:

Husbands of depressed women more frequently than husbands of nondepressed women manifest affective problems (e.g., anxiety, depression, etc.). Moreover, these husbands with problems tend to be from families with significantly higher than average levels of family stresses and chronic problems (problems of health, marriage, and employment).

Initial analyses indicate that children from families with both a depressed mother and a malfunctioning father exhibit more problem behavior than children with a depressed mother and well father.

Significance to Biomedical Research:

This study will advance our understanding of the link between deviance in the husbands of depressed women and elevated rates of risk for maladjustment and psychopathology among their offspring.

Proposed Course:

Coding of interviews and observations relating to father's role in family interaction will proceed.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02207-05 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Affective Rearing Environment: A Comparison of Normal and Depressed Parents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Radke Yarrow	Chief	LDP NIMH
OTHERS:	E. Nottelmann	Statistician	LDP NIMH
	G. Kochanska	Research Psychologist	LDP NIMH
	W.E. Wilson	Research Psychologist	LDP NIMH
	L. Kuczynski	Assoc. Professor	Univ. of Guelph
	J. Richters	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

Univ. of Guelph, Guelph, Ontario, Canada
Division of Research Grants, NIH

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.15

PROFESSIONAL:

.40

OTHER:

1.75

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The affective behaviors symptomatic of the depressed adult are variously expressed in the parent role. How they are expressed by the mother and experienced and responded to by the child are the focus of this project. To be considered, also, is the fact that the child's affective expression, although a response to maternal behavior, may, as well, be an individual contribution that the child brings to the interaction. The naturalistic laboratory paradigm (MH-02144) is the source of data. Measures of affect include: (a) a minute-by-minute rating of emotions and moods of mother and child, (b) a detailed accounting of physical touch and affection, and (c) an experimental intervention in which depressed affect is elicited from the mother in response to a standard stimulus and the subsequent mother-child interaction is recorded.

Analyses are at various stages for these several sets of affective data. Among the findings are: The overall exposure of children to expressed negative mood or emotion of the mother is 17%, 11%, and 6% interaction time with unipolar, bipolar, and normal mothers, respectively. These mean percentages far underestimate the variability related to maternal diagnoses. There are extremes in the depressed groups in which 80 to 90% of the minutes are coded in negative affect. High frequencies on negative affect in the children (the outliers) tend also to be in the offspring of depressed mothers. Children of the unipolar mothers, more than other children, become involved in the mothers' sadness. In such interactions the young child's empathy for the mother and his/ her caregiving responses appear to be exploited. Affect and touch were investigated in the interchange of mothers and their 2 to 3 year olds. Touch is initiated more often by the mother (75% of the occurrences) than the child. The major functions of mothers' touch is to monitor and regulate child behavior and to give affection. The young child's initiations of touch are predominantly to be physically close to (in contact with, leaning on) the mother.

Project Description:

The affective behaviors symptomatic of the depressed adult are variously expressed in the parent role. How they are expressed by the mother and experienced and responded to by the child are the focus of this project. To be considered, also, is the fact that the child's affective expression, although a response to maternal behavior, may, as well, be an individual contribution that the child brings to the interaction. In what respects do affective interactions of mothers and children differ? What are the characteristics of mother and child and relationship characteristics that influence affective behaviors of mother and child?

Methods and Major Findings:

The naturalistic laboratory paradigm (MH-02144) is the source of data. Measures of affect include: (a) a minute-by-minute rating of emotions and moods of mother and child, (b) a detailed accounting of physical touch and affection, and (c) an experimental intervention in which depressed affect is elicited from the mother in response to a standard stimulus and the subsequent mother-child interaction is recorded.

Analyses are at various stages for these several sets of affective data. Among the findings are: (a) The overall exposure of children to expressed negative mood or emotion of the mother is 17%, 11%, and 6% interaction time with unipolar, bipolar, and normal mothers, respectively. These mean percentages far underestimate the variability related to maternal diagnoses. There are extremes in the depressed groups in which 80 to 90% of the minutes are coded in negative affect. (b) High frequencies on negative affect in the children (the outliers) tend also to be in the offspring of depressed mothers. (c) In a standard situation in which mothers are asked to convey sadness, a subgroup of severely unipolar depressed mothers represent their sadness in exaggerated terms, and often redefine the situation by placing responsibility on their children. Children of the unipolar mothers, more than other children, become involved in the mothers' sadness. In such interactions the young child's empathy for the mother and his/her caregiving responses appear to be exploited. (d) Severely depressed (unipolar) mothers who express sadness, anxiety, downcast mood frequently and for long periods of time, interact with the child with intense and prolonged physical contact and affection, especially in stressful circumstances. (e) Touch, a communication that has significant effects on the development of the infant, has not been examined as a variable in the mother-child relationship at later ages. In interchange of mothers and their 2- to 3-year-olds, touch is initiated more often by the mother (75% of the occurrences) than the child. The major functions of mothers' touch is to monitor and regulate child behavior and to give affection. The young child's initiations of touch are predominantly to be physically close to (in contact with, leaning on) the mother. At the present level of group analysis variables of mothers' touch do not differ by mothers' diagnoses. In preliminary analyses, daughters of unipolar mothers initiate more affectionate contact with mother than daughters of the other groups. Among the sons, there are no group differences.

Significance to Biomedical Research:

The offspring of depressed parents are at risk for the development of affective disorders; it is assumed that both genetic and environmental factors contribute to this process. This study offers evidence of specific childrearing conditions that foster development of affective disturbance in young children. The findings are relevant to theories of depression and to issues of prevention.

Proposed Course:

Parallel data have been obtained in the observations at the time of the follow-up three years later. These data will be analyzed along with the earlier assessments. Continuities and discontinuities in the environment and in the development of the child will be examined. Manuscripts are in preparation.

Publications:

Radke-Yarrow M, Richters J, Wilson WE. Child Development in a network of relationships. In: Hinde R, Stevenson-Hinde J, eds. Individuals in a network of relationships. Oxford: Oxford University Press, 1988;48-67.

Radke-Yarrow M, Kochanska G. Anger in young children. In: Stein NL, Leventhal B, Trabasso T, eds. Psychological and biological approaches to emotion. Hillsdale, NJ: Lawrence Erlbaum Press, in press.

Radke-Yarrow M, Belmont B, Nottelmann E, Bottomly L. Young children's self-conceptions: origins in the natural discourse of depressed and normal mothers and their children. In: Cicchetti D, Beeghly M, eds. The development of the self during the preschool years. New England: Cambridge University Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02229-04 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vocalic Analysis of Natural Discourse in Well and Depressed Mothers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. Sherman Research Psychologist LDP NIMH
OTHERS: Z. Breznitz Assistant Professor Univ. of Haifa,
Israel

COOPERATING UNITS (if any)

University of Haifa

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Affective Development

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.30

PROFESSIONAL:

.05

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this set of studies is to examine the patterning of natural discourse of well and depressed women and their young children, and the alternate environmental supports available to young children from their older siblings. The procedural details of the study were reported in Z01 MH 02229-01. The observed differences in amount of vocalization in the mothers' behaviors with their 2- to 3-year-olds were interpreted as an indication that the two groups of children are exposed to very different patterns of socialization. The offspring of depressed women are thus learning both to keep social interaction to a minimum and to be overreactive to even mild stresses.

A question raised by these results concerns the possible long-term sequelae for the children of depressed mothers exposed to this aberrant patterning of communication with their mothers. Conversation between mother and child, and sibling and child are now being examined. Total amount of vocalic behavior of each member of the dyad and the time to initiate vocalization after the other member of the dyad has ceased vocalizing will be recorded. Analysis was discontinued temporarily during this past year.

Project Description:

The purpose of this set of studies is to examine the patterning of natural discourse of well and depressed women and their young children, and the alternate environmental supports available to young children from their older siblings.

The procedural details of the study were reported in Z01 MH 02229-01. The observed differences in amount of vocalization in the mothers' behaviors with their 2- to 3-year-olds were interpreted as an indication that the two groups of children are exposed to very different patterns of socialization. The offspring of depressed women are being taught both to keep social interaction to a minimum and to be overreactive to even mild stresses.

A question raised by these results concerns the possible long-term sequelae for the children of depressed mothers exposed to this aberrant patterning of communication with their mothers. Are the patterns of interaction seen when the children were 3 years of age stable over time? A second is whether the children have alternative sources of social and cognitive support. Conversation between mother and child, and sibling and child is being examined at the time of follow-up. Total amount of vocalic behavior of each member of the dyad and the time to initiate vocalization after the other member of the dyad has ceased vocalizing will be recorded. Analysis was halted temporarily during this past year.

Significance to Biomedical Research:

Dialogue between mother and child is a basic process by which children are socialized and through which children practice aspects of their social and cognitive behavioral systems. Past analyses have demonstrated that depressed mother-child dyads differ from healthy mother-child dyads in certain aspects.

Proposed Course:

Data analysis will be resumed.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02231-04 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological-Behavioral Relations in Early Adolescence

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.D. Nottelmann	Statistician	LDP NIMH
OTHERS:	E.J. Susman	Guest Researcher	LDP NIMH
	G.I. Germain	Research Psychologist	LDP NIMH
	L.D. Dorn	Guest Researcher	LDP NIMH
	G.P. Chrousos	Senior Investigator	DEB NICHD
	G.G. Cutler, Jr.	Senior Investigator	DEB NICHD
	D.L. Loriaux	Chief	DEB NICHD

COOPERATING UNITS (if any)

Developmental Endocrinology, NICHD

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.58

PROFESSIONAL:

.48

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Interrelations of markers of pubertal development and adolescent adjustment and behavior are investigated cross-sectionally and longitudinally. Participants are 9- to 14-year-old boys and girls and their parents. The adolescents and their parents are seen three times, six months apart. The markers of pubertal development include serum hormone levels (gonadotropins, sex steroids, and adrenal androgens), pubertal stage (Tanner criteria), and height and weight. Psychological assessments of the adolescent include adolescent- and parent-report of behavior problems and competencies. Behavioral observations of parents and their adolescent in family interactions also were made. Findings based on behavioral observations suggest that adolescents' aggressive behaviors with parents represent trait-like as well as problem behavior more so for boys than for girls. Longitudinal hormone data analyses indicate that, although the general maturational course for puberty-related hormone levels is a gradual rise, many children show substantial decreases in hormone levels from one time of assessment to the next. Decreases often followed large increases. This pattern of changes is being examined in terms of potential disequilibrating effects on emotional and behavioral functioning. Other analyses indicate that, in addition to the direct association between hormone level and stage of pubertal development, hormone levels appear to be associated with rate of pubertal development. To obtain an index of general hormone change, principal components analyses were carried out. Results indicate that pubertal hormone status in adolescent boys and girls can be indexed reliably by two factors, a gonadal factor and an adrenal factor.

Project Description

Interrelations of endocrine, pubertal stage, and physical growth indices of pubertal development and adolescent adjustment and behavior are investigated cross-sectionally and longitudinally across three times of measurement at six-month intervals.

Methods and Findings

The longitudinal sample consists of 54 boys and 49 girls, 9 to 14 years of age, and their parents. Pubertal development measures include serum hormone levels, height and weight, and pubertal stage (Tanner criteria). Psychological measures include adolescent- and parent-report of the adolescent's behavior problems and competencies. Behavioral observations of parents and their adolescent in family interactions also were made. Details of measurement have been given in reports of this project in previous years (MH 02231-03).

Findings reported earlier suggested that the aggressive behavior of boys is more likely than the aggressive behavior of girls to reflect endogenous factors, and, conversely, that girls may be more responsive to environmental influences than boys. This hypothesis was supported in a further study. Adolescents' aggressive behaviors with parents in direct observations correlated with the more trait-like assessments from parent- and self-reports. This was the case more so for boys than for girls. The findings also suggest that increased expression of assertive forms of aggression is a developmental phenomenon for boys.

Longitudinal evaluation of hormone level changes across the 12-month period of the study, assessed by correlational analyses, indicates overall stability in hormone levels: for boys, relatively high stability ($r \geq .70$) in androgen levels (testosterone, dehydroepiandrosterone and its sulphate, and androstenedione); moderate stability ($r < .70$ and $\geq .50$) for follicle stimulating hormone levels, and low stability ($r < .50$) for luteinizing hormone and estradiol levels; for girls, moderate to high stability ($r \geq .50$) in adrenal hormone levels (dehydroepiandrosterone and its sulphate and androstenedione), low stability ($r < .50$) in gonadotropin (luteinizing hormone and follicle stimulating hormone) and estradiol levels, and no stability in testosterone levels.

Change in hormone levels was examined on a case-by-case basis in standardized change scores. Although the general maturational course for these hormone levels is to rise gradually through puberty, in many cases there were substantial decreases in hormone levels from one time of assessment to the next. Often, decreases across the second 6-month period followed large increases in level across the first 6-month period. The decreases may reflect counterregulatory processes that compensate for initial "overshooting" of hormone secretion. Data on adolescents whose hormone levels reflect this pattern will be examined for potential disequilibrating effects on emotional and behavioral functioning.

There is not only a direct association between hormone levels and stage of pubertal development; hormone levels appear to be associated also with the subsequent rate of physical pubertal development. Initial hormone levels within Stage 1, 2, and 3 pubertal cohorts were higher for those individuals who advanced in pubertal stage across the next year than for individuals who

remained at the same stage. Furthermore, those who advanced more than one pubertal stage across the year had higher hormone levels initially than members of the same cohort who advanced only one stage.

The possibility was explored of developing an index of overall hormonal status that can be used in analyses of hormone-behavior relations. For boys, principal components analyses yielded two very distinct hormone factors (accounting for 65% of the variance) that replicate at the three times of assessment: a gonadal factor luteinizing hormone, follicle stimulating hormone, testosterone, and estradiol) and an adrenal factor (dehydroepiandrosterone and its sulphate and androstenedione). For girls, two similar hormone factors (accounting for 67% of the variance) that replicate at the three times of assessment were obtained but their factors were somewhat less distinct, with testosterone and androstenedione loading ($>.50$) on both factors.

Significance to Biomedical Research

Increases in behavior problems during adolescence have been noted extensively. Findings from this study provide systematic documentation of behavior change during puberty and begin to clarify how such problems may be related to developmental status and rate of pubertal change in early adolescence.

Proposed Course

Work continues on interrelations between pubertal change and competencies and dysfunctions in the psychological domain. Relations between the two sets of measures are being examined at two periods of measurement to determine if the relations change as the boys and girls mature. A longitudinal examination is under way of interactions of adolescents with parents in relation to pubertal change.

Publications

Inoff-Germain G, Arnold GS, Nottelmann ED, Susman EJ, Cutler GB, Jr, Chrousos GP. Relations between hormone levels and observational measures of aggressive behavior of young adolescents in family interactions, *Dev Psychol* 1988;24(1):129-139.

Inoff-Germain G, Nottelmann ED, Arnold GS, Susman EJ. Adolescent aggression and parent-adolescent conflict: relations between observed family interactions and measures of the adolescents' general functioning. *J of Early Adolesc*, in press.

Nottelmann ED, Inoff-Germain G, Susman EJ, Chrousos GP. Hormones and behavior at puberty. In: Bancroft J, ed. *Adolescence and puberty*. Oxford: Oxford University Press, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02233-03 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Development of Guilt : Language, Emotions, and Behavior

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Zahn-Waxler

Chief, Section on
Child Behavior Disorders

LDP/NIMH

Others: K. Barrett
G. Kochanska
P. ColeAssistant Professor
Research Psychologist
Senior Staff FellowUniv. of Wyoming
LDP/NIMH
LDP/NIMH

COOPERATING UNITS (if any)

University of Wyoming, Laramie, Wyoming

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.59

PROFESSIONAL:

.55

OTHER:

.04

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Certain mental disorders are characterized by atypical patterns of guilt (e.g., excesses of guilt in depression and deficiencies in sociopathy). Few data are available to indicate why some individuals develop the capacity to maintain healthy, appropriate levels of responsibility, while others experience excesses or deficiencies in guilt at levels that seriously compromise their well-being. Three samples of children have been studied. In studies 1 and 2, two- to three-year-old children are observed in structured situations in which a problem or mishap is staged and the child's tendency to assume responsibility is assessed. Findings from both studies indicate that a majority of children engage in reparative behaviors by age two. Many children with a depressed mother assume disproportionate levels of responsibility. In study 3, guilt is assessed in children between the ages of 5 and 8 (Z01 MH 02155 LDP) in a semi-structured projective test situation and in a psychiatric interview. Here too, the younger children of depressed mothers show high levels of guilt and involvement in others' problems. But these same children fail to show the increases with age in adaptive expressions of guilt that are expected and found in children of well mothers. Proband children also show more distorted (e.g., extreme, bizarre, violent, disguised) forms of guilt.

Project Description

Certain mental disorders are characterized by atypical patterns of guilt (e.g., excesses of guilt in depression and deficiencies in sociopathy). Few data are available to indicate why some individuals develop the capacity to maintain healthy, appropriate levels of responsibility, while others experience excesses or deficiencies in guilt at levels that seriously compromise their well-being. This research focuses on patterns of adaptation and maladaptation in the development of guilt in children. Families and children are selected for characteristics (e.g., gender, parental depression) that, on theoretical grounds, might be expected to contribute to differential development of guilt and different forms of expression.

Methods Employed and Major Findings

Three samples of children have been studied using varied instruments to assess cognitive, affective and behavioral signs of guilt. In studies 1 (N = 100) and 2 (N = 50) two- to three-year-old children are observed in structured situations in which a problem or mishap is staged (e.g., a toy breaks, juice spills, mother looks sad, child accidentally hits and hurts another), and the child's tendency to become involved and assume responsibility is assessed. A majority of children in both studies engage in reparative behaviors by age two. The reactions of children of depressed and well mothers are compared in study 1. Many children with a depressed mother assume disproportionate levels of responsibility (guilt). That is, children of depressed mothers are more likely than children of well mothers to try to alleviate the mothers' distress. In study 2 (a normal volunteer sample), children's reparative, aggressive and avoidant behaviors and their regulation of affect are investigated. Differences will be examined in relation to maternal personality characteristics. In study 3, children's (ages 5 and 8) (Z01 MH 02155) responses to pictures of distress and conflict again show differences relating to maternal depression. In children of well families, adaptive expressions of guilt and responsibility increase with age, but in children of depressed mothers, this developmental change does not occur. Exaggerated, extreme, or bizarre expressions of guilt are most commonly found in children of depressed mothers. Guilt is related to different emotions in children of well and ill mothers (i.e., to empathy in children of well mothers and to hostility in children of depressed mothers).

Significance to Biomedical Research and the Program of the Institute

Emotions of guilt and related expressions of low self-esteem and worthlessness are major features of psychopathology. Knowledge of the developmental course, the conditions under which different forms of guilt develop, and the circumstances under which guilt begins to become linked with other affective and social maladaptations will contribute an understanding of how these aspects of psychopathology evolve. Basic research on development of guilt in children begins to provide this information.

Proposed Course

Data collection has been completed, and most of coding. One manuscript has been completed and a chapter written. Other publications are projected.

Publications

Zahn-Waxler C, Kochanska G. The development of guilt. In: Thompson R, ed. 1988 Nebraska Symposium on Motivation and Social-emotional Development, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02234-03 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Infants of Chronically Depressed and Normal Parents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.D. Nottelmann	Statistician	LDP NIMH
OTHERS:	K. Suter	Guest Researcher	LDP NIMH
	J. Stilwell	Research Nurse Practitioner (Psychiatric)	LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.41

PROFESSIONAL:

.11

OTHER:

.30

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Research has shown that children of depressed parents are at risk for development of affective illness, but little is known about the effects of parental affective psychopathology on infants during the first eighteen months of life. This study examines early affective regulation, attachment behavior, and patterns of mother-infant interaction in infants of two groups of parents: (1) depressed mothers and well fathers, and (2) both parents without psychiatric illness. Parents are screened by structured psychiatric interview (SADS-L) and also complete questionnaire measures of marital adjustment (DAS). Infants and their mothers are observed and videotaped in a set of standard situations in a homelike laboratory setting on two occasions five months apart, beginning when the infants are age 3 months, 8 months, or 13 months. Data collection has been completed. The findings on attachment replicate an early study in this laboratory of a higher rate of insecure attachment in 1 1/2 year olds of affectively ill mothers, than in children of well mothers. Data coding on other aspects of infant and mother behavior is in progress.

Project Description

Research has demonstrated that children of depressed parents are at risk for development of affective illness themselves; considerable attention has been focused on latency age and adolescent offspring at risk for depression. More recently, this laboratory has examined toddlers and latency age children in families with parental history of affective disorder or no psychiatric diagnosis and has found a number of differences in children and parenting styles between different parental diagnostic groups (see Z01 MH 02144). The purpose of the current project is to look for possible early precursors in infants at risk for later affective illness. Infants of two groups of parents are included: (1) depressed mothers and well fathers, and (2) both parents without psychiatric illness. Attachment behavior, early affective regulation, and patterns of mother-infant interaction are examined.

Parents are screened by structured psychiatric interview (SADS-L). Infants are seen with their mothers in a homelike laboratory setting for two sessions five months apart beginning at one of three ages: 3 months, 8 months, or 13 months. The 13-month group includes 9 depressed mothers and 10 control mothers; the 8-month group, 14 depressed mothers and 9 control mothers; and the 3-month group, 7 depressed mothers and 5 control mothers. Mother-infant interaction is observed and videotaped in a set of standard situations, including introduction to a new environment, free play, introduction of a stranger, brief separation, feeding, and developmental testing. Situations are adapted to be appropriate for each age group. Parents also complete a questionnaire measure of marital adjustment (Dyadic Adjustment Scale).

Data are being coded and analyzed for a number of variables, including attachment classification, social referencing, affective regulation, early signs of mastery motivation, and patterns of mother-infant interaction. Comparisons will be made between diagnostic groups as well as examined for developmental and individual differences.

A high degree of insecure attachments was found in infants of depressed mothers at 13 months (61%) and at 18 months (67%). Although the control group also showed high insecurity (58%) at initial 13-month assessments, at 18 months it resembled normal samples reported on elsewhere, with 70% secure attachment.

Significance to Biomedical Research

Little is known about the effects of maternal depression on infants. This study will provide data on this issue as well as attempt to identify possible precursors of later difficulties in a population at risk for later affective disorder.

Proposed Course

Data collection has been completed. Data are currently being coded. Data analysis and the preparation of one manuscript are to be completed this year. This is a final report. The principal investigator has left the Laboratory.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02297-03 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Generosity and Sharing in Children of Normal or Affectively Disturbed Parents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F.R. Ascione	Guest Researcher	LDP NIMH
OTHER:	M. Radke-Yarrow	Chief	LDP NIMH
	C. Zahn-Waxler	Chief, Sec. on Child Behavior Disorders	LDP NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been discontinued since the former guest researcher has been unable to give more time to this work. Data are being incorporated in 02372.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02361-02 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Relation Between Self- and Teacher-Reports of Social-Emotional Adjustment

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. Sherman Research Psychologist LDP NIMH
OTHER: D. Pellegrini Professor Catholic Univ.

COOPERATING UNITS (if any)

Catholic University

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Affective Development

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.30

PROFESSIONAL:

.05

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The question addressed by this study is the extent to which the children's reports of negative affective states, poor social relationships with family members and/or peers, and problems in school correspond to the child's level of adaptation (academic performance, mood regulation, and social relations with peers and adult authority) in the school setting. A secondary question is the extent to which patterns of adaptation in the school context relate to parental psychopathology.

One hundred and twenty children (the entire group of older siblings in the NIMH Longitudinal Study, Annual Report Z01 MH 02207-05) 8 to 11 years of age were administered the Child Assessment Schedule (CAS), a structured psychiatric interview. In addition, the children's teachers filled out the Achenbach Child Behavior Checklist - School Version. On the Achenbach the teachers are asked to report on the children's ability to relate to other children, the children's behavior, and the children's academic achievement.

Project Description:

The question addressed by this study is the extent to which the children's reports on the CAS of negative affective states, poor social relationships with family members and/or peers, and problems in school correspond to the child's level of adaptation (academic performance, mood regulation, and social relations with peers and adult authority) in the school setting.

As with adults, children's self-reports and internal states are a significant aspect of defining psychopathology. In addition, though, it is important to establish whether these feeling states translate into impairments in life functioning.

Methods Employed:

One hundred and twenty children (the 8- to 11-year-olds in the Childrearing Study, Z01 MH 02207) were administered the Child Assessment Schedule (CAS), a structured psychiatric interview. In addition, the children's teachers filled out the Achenbach Child Behavior Checklist - School Version, a report on the children's ability to relate to other children, the children's behavior, and the children's academic achievement.

Significance to Biomedical Research:

A fundamental issue in understanding the developmental course of affective illness is to identify significant premorbid signs and/or symptoms. The contribution of this study will be to indicate the relation for school-age children, between their symptom picture as revealed in their major work and social setting and their symptom picture as revealed via self report.

Proposed Course:

Analyses will not begin until all the data have been collected.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02362-02 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physical/Neurological Development in Children of Healthy and Depressed Mothers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. Sherman

Research Psychologist

LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Affective Development

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been incorporated into project Z01 MH 02361-02.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02363-02 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Information-Processing Deficits in Schizophrenic Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. Sherman

Research Psychologist

LDP NIMH

OTHER: R. Asarnow

Associate Professor

UCLA School of
Medicine

COOPERATING UNITS (if any)

UCLA School of Medicine

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.35

PROFESSIONAL:

.10

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this study, based on the Feature Integration Theory of Attention by Treisman and Gelade, the ability of the schizophrenic child to recognize the task relevance of, and engage in, serial and parallel modes of visual search is examined. Schizophrenic children were as competent as MA-matched control children in their use of a parallel visual search strategy and a serial visual search strategy, and in their recognition of the situations under which each is the optimal strategy; they had a significantly greater start-up time than the MA-matched controls in the initiation of their search strategies, greater than would be predicted on the basis of merely a motor delay in button pushing. Results suggest that the schizophrenic individual does not have a specific information processing deficit, but rather a global deficit in time to initiate the operation of any information processing strategy, be it an automatic strategy or an attention demanding strategy.

Project Description:

This project was designed to investigate cognitive impairments in schizophrenic children. Based on the Feature Integration Theory of Attention by Treisman and Gelade, this study sought to examine the ability of the schizophrenic child to recognize the task relevance of and engage in serial and parallel modes of visual search.

Methods and Findings:

For details of sample see methods and findings, Z01 MH 02363-01. Initial findings were reported last year.

Significance to Biomedical Research:

Research on schizophrenic children provides information on a sample that is less likely than adult samples to have suffered additional impairments due to prolonged drug therapy or institutionalization. In addition, schizophrenic children are probably a more homogeneous group than adult schizophrenics, and are probably a group with a higher genetic loading for the illness. Thus, research on schizophrenic children offers a potentially purer sample in which to study cognitive impairments associated with schizophrenia.

Proposed Course:

Reanalyses were undertaken this past year and will be completed before submitting the manuscript for publication.

This is a final report.

Publications:

Mundy P, Sigman M, Ungerer J, Sherman T. Nonverbal communication and play correlates of language development in autistic children. J of Autism and Dev Disorders 1987;17(3):349-364.

Asarnow R, Granholm E, Sherman T. Span of apprehension in schizophrenia. In: Nasrallah HA, Zubin J, Steinhauer S, Gruzeliier JH, eds. Handbook of schizophrenia, vol 4. Experimental psychopathology, neuropsychology and psychophysiology. New York: Elsevier Science Publishers, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02364-02 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Follow-up Investigation of Offspring of Bipolar Parents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Zahn-Waxler Chief, Section on LDP/NIMH
Child Behavior Disorders

Others: L. Cytryn Guest Researcher LDP/NIMH
A. Mayfield Social Science Analyst LDP/NIMH
D. McKnew, Jr. Guest Researcher LDP/NIMH
M. Radke-Yarrow Chief LDP/NIMH
Y. Davenport Social Worker Chestnut Lodge

COOPERATING UNITS (if any)

Chestnut Lodge, Rockville, Maryland

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.11

PROFESSIONAL:

.10

OTHER:

.01

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

A follow-up study was conducted of seven children of severely affectively-ill parents. The children had been studied in infancy. One parent in each of the families was a bipolar depressed patient; in five of these families, the other parent had a diagnosis of unipolar depression, a sixth was substance abusing. At 1 1/2 years the children had insecure attachment relationships with their mothers, problems in regulation of affect, and disturbances in empathy and aggression. Psychological and psychiatric assessments of the children were conducted at 5 and at 6 years to determine whether problems identified earlier were transitory or persistent and whether other disturbances had developed

On the follow-up, the provand children differed from a control group on many dimensions of functioning. In a psychiatric interview, they reported more fears, worries, depressive feelings and distortions in self-image. Their mothers reported a high incidence of externalizing, as well as internalizing symptoms in the children. Deficits in empathy and non-assertive strategies for resolving conflicts also were identified.

Project Description

This research is a follow-up evaluation of high-risk children who had been studied as infants. They are the offspring of bipolar parents (annual report # Z01 MH 02155). Problems in relationship formation, affect regulation, and coping with stress had been identified in the children when they were infants and toddlers. The purpose of the current research was to determine whether the early problems reflected transitory or persistent diagnosable disturbance.

Methods Employed and Major Findings

Seven male children with a severely ill manic-depressive parent were seen in infancy and again at ages five and six. Four of the bipolar parents were female and three were males who had been inpatients at NIMH. Diagnoses of bipolar affective disorder were determined on the basis of SADS-L interviews, using RDC criteria. Five of the seven spouses were diagnosed with unipolar depression and one with substance abuse and war neurosis. All of the bipolar parents had a familial history of affective disorder. The current family environments were characterized by disorganization, unpredictability, alienation, and weak social support systems. There was a comparison group of 11 children with nondepressed parents.

At age five, the children were observed in the laboratory in procedures chosen to assess the areas of difficulty identified earlier in development. The children were observed in interaction with a playmate, under pleasurable and under mildly stressful conditions. They were given structured tests developed to assess empathy and conflict resolution strategies. At age six, the children returned for a standard psychiatric interview, the Childhood Assessment Schedule. DSM-III diagnoses were determined, based on the child's responses to the psychiatric interview and data obtained from the mother on the Achenbach Child Behavior Check List.

Children in the proband sample reported more fears, worries, and depression than control children on the Childhood Assessment Schedule and they also scored higher on a subset of items of suicidal ideation and/or proneness to self-injury. Their mothers reported them to be high on externalizing as well as internalizing symptoms. Psychiatric diagnoses were more frequent and of a more serious nature in proband than in control children. Some of the proband children showed continuity over time in the specific symptoms of disturbance; others showed changes in the types of problems. As a group, proband children showed atypical patterns of empathy and social problem solving.

Significance to Biomedical Research and the Program of the Institute

The literature on children with a bipolar parent has yielded mixed findings. In some studies these children are described as super competent; in others, many problems are reported. It is important to begin to identify the origins of these differences in order to understand the developmental course in these children, and more effectively to plan prevention and intervention strategies. This research provides information about the frequency, nature and severity of symptoms in a group of children for whom there is high genetic loading for

affective disorder, as well as the presence of highly stressful family environments. The present research helps to identify some of the specific vulnerabilities in these children.

Proposed Course

A manuscript based on this work has been accepted for publication. This is a final report.

Publications

Zahn-Waxler C, Mayfield A, Radke-Yarrow M, McKnew D, Cytryn L, Davenport Y. A follow-up investigation of offspring of bipolar parents, Am J Psychiatry 1988; 145:506-9.

Pierrehumbert B, Iannotti R, Cummings E, Zahn-Waxler C. Social functioning with mother and peer at two and five years of age: The influence of attachment. Int J of Behav Dev, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02365-02 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Psychobiological Effects of Sexual Abuse

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F.W. Putnam Staff Psychiatrist LDP/NIMH

Other: P.K. Trickett Guest Researcher LDP/NIMH

COOPERATING UNITS (if any)

Chesapeake Institute, Kensington, Maryland; Montgomery County Child Protection Unit; Prince Georges County Child Protection Unit; Virginia Child Protection Services, Fairfax County, Virginia

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.72

PROFESSIONAL:

.70

OTHER:

.02

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study focuses on the psychological and biological effects of sexual abuse in female children. Subjects are sexually abused females (6-15 years of age) and a non-abusing parent or guardian. Control subjects are age- and SES-matched. The study uses a multi-method approach to gather information on the psychological and physical development of these children. Methods include: staging physical development, measurement of blood hormone levels, psychological tests and measures on the children and their guardians. Three hypotheses are tested: 1) That sexually abused girls will have a more difficult transition through puberty; 2) That sexual abuse may effect specific hormonal levels and alter the timing and onset of puberty, and 3) That sexually abused children will demonstrate higher levels of dissociation compared to controls.

Project Description:

Objectives: Sexual abuse, largely unrecognized until the late 1970's, is now known to constitute a major form of child maltreatment in the United States. While statistics on the incidence of sexual abuse in children vary widely, even the most conservative reports indicate that this is a major public health problem. The long-term effects of sexual abuse are considerable. Prominent symptoms in adults suffering sexual abuse as children include: depression, suicidality, substance abuse, multiple somatic complaints, and sexual dysfunction. In children, the data are less clear, but frequently reported symptoms include: depression, running away, learning disabilities, self-mutilation, conduct disorders and inappropriate sexual behavior.

This study is the first attempt to prospectively follow a group of sexually abused children in a longitudinal fashion through puberty. Three hypotheses are tested: 1) that puberty exacerbates the impact of sexual abuse on the psychological development of girls; 2) that certain specific behaviors commonly reported in abused children, i.e. aggression and inappropriate sexuality, are associated with alterations in adrenal androgenic and gonadal hormonal levels; and 3) that dissociative behavior, a psychophysiological response to extreme trauma, is increased in sexually abused girls compared to matched controls.

Methods Employed:

This study uses a convergence or cross-sequential prospective design. Psychological development is assessed across two broad domains; 1) indicators of competence and coping with the developmental tasks of puberty and 2) the presence of psychiatric symptoms and behavioral problems. Physical development is assessed using Tanner Staging and serial hormonal levels. Dissociative capacity is measured using standardized hypnosis scales, a child dissociation checklist and the Dissociative Experiences Scale (DES).

Significance to Mental Health Research:

This study is the first of its kind in that it seeks to prospectively document, in a longitudinal fashion, the impact of sexual abuse in children. Three mechanistic hypotheses are tested to seek to explain the frequently observed clinical findings.

Proposed Course of the Project:

This project is partially funded by a three year grant from the W.T. Grant Foundation. Data are being collected on sexually abused children and their age- and SES-matched controls. We are currently seeing 1-2 families per week and plan to collect about 100 new families (50 subject and 50 controls) in year 1. Serial follow-up will begin on these first families in year 2 and an additional 100 families will be added. Serial follow-up will continue on all families for a total of two years.

Publications:

Putnam FW The Disturbance of 'Self' in Victims of Childhood Sexual Abuse.
In Klufit RP, ed. Incest Related Psychiatric Syndromes of Adult
Psychopathology. Washington, D.C., American Psychiatric Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02366-02 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Psychophysiology of Multiple Personality Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F.W. Putnam	Staff Psychiatrist	LDP NIMH
OTHERS:	R.M. Post	Chief	BPP NIMH
	D. Weinberger	Psychiatrist	CBDB NIMH
	T. Zahn	Psychiatrist	LPP NIMH
	O. Devinsky	Psychiatrist	LPP NIMH
	J. Grafman	Psychiatrist	CES NINCDS
	N. Hall	Psychiatrist	George Wash. Univ.

COOPERATING UNITS (if any)

George Washington University, Laboratory of Psychology and Psychopathology,
Biological Psychiatry Branch, Clinical Brain Disorder Branch

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.80

PROFESSIONAL:

.75

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project seeks to measure psychophysiological differences that have been reported to exist across the alter personality states of patients with multiple personality disorder (MPD). Alter personalities of MPD patients are studied on a range of measures including: spontaneous EEG, auditory and visual evoked potentials, cerebral blood flow, continuous catheter venous sampling, psychological tests and measures of autonomic nervous system activity such as galvanic skin response, skin temperature, pulse and respiration. Simulating normal control subjects serve as a comparison group.

Project Description:

Objectives: This project seeks to ascertain and quantify any differential psychophysiological effects across alter personality states of MPD subjects.

Methods Employed: A variety of psychophysiological measures have been used in this project. These included averaged evoked potentials to light and sound; galvanic skin response and other autonomic measures; xenon-inhalation cerebral blood flow; continuous telemetry EEG with implanted sphenoidal electrodes; and circulating immune factors drawn from continuous venous sampling by indwelling catheters. Changes across alter personality states are statistically compared to those produced by normal control subjects simulating behavioral and personality changes.

Past Findings:

Studies from this project indicate that MPD patients produce changes in visually evoked potentials and spontaneous EEG that can not be duplicated by age- and sex-matched simulating control subjects.

New Findings:

Round-the-clock EEG telemetry for periods up to two weeks has conclusively demonstrated the independence of dissociative symptoms and epileptic phenomena in six MPD subjects.

Significance to Mental Health Research:

The demonstration of the independence of dissociative and epileptic phenomena is important because a number of clinical articles have suggested that anticonvulsant medication may be useful in the treatment of MPD. Our data would suggest that any beneficial clinical effects of anticonvulsant medication in MPD patients is not due to an anti-epileptic response per se.

Proposed Course:

On this project, I will continue to collect data on a range of psychophysiological measures across alter personality states in MPD subjects and simulated control states. Multiple personality disorder provides a unique model of the interaction of psychological states and psychosomatic phenomena.

Publication:

Putnam FW: The switch process in Multiple Personality Disorder and other state-change disorders. Dissociation 1:, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02367-02 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Clinical Phenomenology of Multiple Personality Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F.W. Putnam

Staff Psychiatrist

LDP/NIMH

Other: R.J. Lowenstein

Chief

Dept. Psy., UCLA

R.M. Post

Chief

BPP/NIMH

COOPERATING UNITS (if any)

UCLA

Biological Psychiatry Branch

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.32

PROFESSIONAL:

.30

OTHER:

.02

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Clinical syndrome of Multiple Personality Disorder (MPD) is a major dissociative disorder that is being increasingly diagnosed in victims of severe child abuse. While the disorder has been recognized for over 200 years, it is poorly characterized and little is known about its long term outcome. At present, there are an estimated 7000 diagnosed cases in the U.S. We have systematically collected data on over 200 cases in treatment in the United States. Our results indicate that MPD is a clinical syndrome with core features of dissociative and depressive symptoms in individuals with a history of child abuse occurring between ages 2 to 14 years of age. There appear to be important differences between males and females on a number of features.

Project Description:

Objectives -- Until the last decade, MPD was considered to be an extremely rare condition. With the inclusion of MPD in the DSM-III in 1980, many more cases were recognized, until currently over 7000 identified patients exist in the U.S. A number of institutions are developing specialized treatment settings to handle these perplexing patients. The purpose of this project has been to characterize the disorder, determine the range of clinical presentations and the responses of patients to standard clinical interventions.

Methods Employed:

A 386-item standardized questionnaire designed to collect detailed information on a single case, was initially distributed to clinicians known to be interested in the disorder. A national sampling was obtained of patients meeting DSM-III criteria for MPD. Subsequent samples have focused on specific subgroups of MPD patients, including male cases, MPD patients with concurrent diagnoses of eating disorders, adolescents and children.

Major Past Findings:

The overall patient population currently in treatment for MPD was found to be predominately female (>90%) with a mean age at time of sampling of 35.8 years. MPD patients present with multiple psychiatric and medical symptoms (mean number of symptoms 18.5). The most common psychiatric symptoms include depressed mood, mood swings, sleep disturbances, sexual dysfunction and suicidality. The average number of alter personality states was 13.3 with a standard constellation of types of alters found across most patients. A history of significant childhood trauma was found in 97% of cases and there was a correlation between the number of different types of trauma experienced by the individual and the number of alter personality states ($r=.39$, $p < .0001$).

New Findings:

Data from this study are being analyzed for profiles related to treatment outcome. In addition, several subsamples are being examined to examine the interrelationship between MPD and other psychiatric disorders. Data on male patients indicates that they are highly likely to have histories of major aggression and violence compared to female cases.

Significance to Mental Health Researchers:

This projects represents the first systematic attempt to conduct a large scale study of independent cases of MPD. The data from this study are being used to construct profiles of MPD patients to aid in the diagnosis and to determine prognostic indicators and response to standard psychiatric therapies.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02368-02 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Dissociative Experiences Scale (DES)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F.W. Putnam

Staff Psychiatrist

LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Childhood Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.70

PROFESSIONAL:

.60

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Dissociative Experiences Scale (DES) is a short, self-administered questionnaire that reliably and validly discriminates patients with major dissociative psychopathology from other psychiatric patients and normal control groups. The DES has been included in a large number of studies in the U.S. and in Europe. French, Dutch and Cambodian translations are in use. There are several papers in press that have replicated our original findings using U.S. and Canadian patient samples. Current research using this instrument is focused on symptoms of dissociation in epileptic and eating disorder cohorts at the NIH.

Project Description:

Objective -- The phenomenon of dissociation is a complex psychophysiological process that appears to contribute to the psychopathology of a spectrum of psychiatric disorders, primarily those associated with exposure to psychological trauma. The clinical recognition and study of dissociative phenomena have been severely limited by the lack of a reliable and valid instrument to identify and quantify these symptoms. In collaboration with Eve B. Carlson, Ph.D., currently at University of North Carolina, Greensboro, NC, we have developed a short, self-administered, computer-scored questionnaire that yields an overall index score and three subscale scores that reliably quantify dissociative experiences.

Methods Employed:

Reliability testing has included both test-retest and split-half measures. Validity testing has consisted of demonstrating discriminative validity across a range of diagnostic samples and criterion-referenced validity by a high correlation ($r = 0.62$, $p < 0.01$) with a standardized measure of hypnotizability (Stanford Hypnotic Susceptibility Scale). In addition, two research groups have papers in press that include replications of our original findings across a variety of patient samples.

New Findings:

The DES has been used to investigate two carefully diagnosed samples of epileptic patients. A sample of 71 general and partial seizure patients was collected in collaboration with Orrin Devinsky, M.D. of the NINCDS. These patients scored significantly higher on the DES than an age-matched group of normal controls ($\text{ChiSq} = 14.29$, $p < 0.0002$) but significantly lower than an age-matched group of multiple personality patients ($\text{ChiSq} = 66.44$, $p < 0.0001$). Patients with partial seizures had significantly higher levels of dissociation than those with general seizures ($\text{ChiSq} = 4.47$, $p < 0.038$); while epileptic patients with brain lesions in the dominant hemisphere had significantly more depersonalization than those with non-dominant hemisphere lesions ($\text{ChiSq} = 4.75$, $p < 0.029$). These findings were essentially replicated with a smaller sample of seizure patients studied in collaboration with Richard Loewenstein, M.D., formerly at UCLA Medical School.

In collaboration with Mark Demitrack, M.D. of the Psychoendocrinology Branch, NIMH, IRP, a group of 16 consecutive admissions of eating disorder patients were studied with the DES. As a group, eating disorder patients had significantly higher DES scores than age- and sex-matched normal controls, though they exhibited a bimodal distribution of DES scores. The subgroup with high DES scores had a significantly longer illness course and a trend toward a lower level of mean urinary free cortisol ($r = -.40$, $p < .08$) compared to low scoring eating disorder patients. A larger sample of eating disorder patients is under current investigation.

Significance to Mental Health Research:

Dissociation is believed to play a major role in the psychopathology of a number of psychiatric disorders. While patients with major dissociative pathology may symptomatically appear to have other disorders, e.g. depression or anxiety, they are refractory to the standard treatment for these other disorders. The DES allows clinicians to quickly and simply screen patients for the presence of major dissociative psychopathology and identify psychiatric patients who will respond to treatment for dissociative disorders.

Proposed Course of the Project:

Current studies with the DES include: 1) survey of eating disorder patients; 2) survey of affective disorder patients; 3) survey of borderline personality disorder patients; 4) survey of parents of sexually abused girls. We are looking at the correlation of high levels of dissociation and biochemical abnormalities in studies 1 & 2 above. We will continue to increase our samples of: 1) schizophrenics, 2) post-traumatic stress disorder; 3) panic anxiety disorder; 4) multiple personality disorder; 5) affective disorder; 6) premenstrual tension syndrome; 7) and organic brain syndromes. In addition, a cross-cultural study in collaboration with a number of French psychiatric centers is in progress to examine the role of cultural factors in dissociative symptoms. Two papers on the overlap of dissociative symptoms in epileptic patients are in preparation.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02369-02 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mutual Interpersonal Influences in Families With and Without Affective Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Kochanska

Research Psychologist

LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.10

PROFESSIONAL:

.50

OTHER:

.60

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Processes of mutual interpersonal influence between well and depressed mothers and their 5-year-old children are examined. This is a longitudinal study, following up the sample studied when the children were 1 1/2 to 3 1/2 years old (Z01 MH 02144). The study is focused on difficulties of depressed mothers in controlling their children, and the development of children's competent interpersonal responses to control. Both issues were investigated in the initial study. Two major research questions are addressed: What is the relation between the mother's psychopathology and the processes of interpersonal influence between mother and child, and what are the developmental changes from the time the child was two to when he/she was five years of age? The focus is also on the children as active agents of influence, their strategies directed to the mothers, and the mothers' responses to children's control. These bilateral influence processes are studied by analyzing every episode of control, initiated either by the mother or by the child, during naturalistic interaction. Toddler-age findings, that daughters of depressed mothers were more noncompliant, were also replicated at 5 years. In contrast to the toddler-age findings, when depressed mothers were less assertive than normal mothers, when children were 5 the depressed mothers showed more directness and assertiveness than the well mothers. From the longitudinal comparisons, it appears that in some respects the depressed mothers failed to make appropriate developmental adaptations to their children's increasing capacities of self-regulation. However, in both groups robust developmental changes in maternal strategies, as well as children's responses, were identified.

Project description.

Processes of mutual interpersonal influence between well and depressed mothers and their 5-year-old children are examined. This is a longitudinal study, following up the sample studied when the children were 1 1/2 to 3 1/2 years old (Z01 MH 02144). The study is focused on: difficulties of the depressed mothers in controlling their children, and the development of children's competent interpersonal responses to control. Both issues were investigated in the initial study.

Two major research questions are addressed: What is the relation between the mother's psychopathology and the processes of interpersonal influence between mother and child, and what are the developmental changes from the time the child was two years of age and the time he/she is five? In addition to examining the mothers' interpersonal strategies and children's responses, the focus is also on the children as active agents of influence, their strategies directed to the mothers, and the mothers' responses to children's control. These bilateral influence processes are studied by analyzing every episode of control, initiated either by the mother or by the child, during naturalistic interaction.

Method

The method was described in the Annual Report Z01 MH 02152. A similar approach was adopted here. Ninety minutes of interaction was sampled from videotapes of mother-child interaction to provide comparable time sample. The behavioral coding system used when the children were 5 years old is compatible with the system used when they were toddlers, which provides a possibility for longitudinal assessment.

Findings

In both groups of mothers, there were robust developmental changes. Mothers relied less on strategies based on external reinforcements (both positive and negative), which reflects their recognition of child increased capacity for self-regulation. They also more often used abbreviated commands and hints, reflecting the shared understanding within mother-child dyad, that has developed during their relationship. When children's responses to maternal control were examined, the previously described developmental trends were replicated. Less competent forms of response (passive noncompliance and overt defiance) continued to decrease, while the more advanced forms (direct refusals and negotiations) continued to rise.

In contrast to the findings at the toddler age, when depressed mothers were less assertive than normal mothers, when children were five years of age the depressed mothers showed more directness and assertiveness than the well mothers. When the severity of depression was considered (level of impairment combined with chronicity), the most severely ill group was found to be more direct than the less ill mothers or well mothers. From the longitudinal comparisons, it appears that the depressed mothers failed to make appropriate developmental adaptations to their children's increasing capacities of self-regulation and increased cognitive capacities. In contrast, the well mothers,

while controlling their 5-year-olds, have adopted to a greater extent strategies indicative of their recognition of child developmental progress. Toddler-age findings, that daughters of depressed mothers were more non-compliant, were also replicated at 5 years. The children of mothers who had experienced most severe and chronic depression showed higher level of passive noncompliance than any other group.

Significance for Biomedical Research.

The study will provide further insight into the interactive patterns in families with affective disorder. Day-to-day processes of interpersonal influence may be a significant factor in the emergence of disordered patterns of interaction, and therefore may contribute to the increased risk for the development of psychopathology.

Proposed course.

Analyses will continue to address in greater detail the mothers' and children's influence strategies. Several manuscripts are being prepared for publication.

Publications.

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02370-02 LDP																		
PERIOD COVERED October 1, 1987 through September 30, 1988																				
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Caregiving Patterns in Stressed Families																				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: F. Bridges-Cline</td> <td style="width: 33%;">Guest Researcher</td> <td style="width: 33%;">LDP NIMH</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>OTHERS: W.E. Wilson</td> <td>Research Psychologist</td> <td>DRG NIH</td> </tr> <tr> <td>M. Radke-Yarrow</td> <td>Chief</td> <td>LDP NIMH</td> </tr> <tr> <td>D. Hay</td> <td>Research Psychologist</td> <td>Univ. of London</td> </tr> <tr> <td>T. Cox</td> <td>Psychiatrist</td> <td>Univ. of Liverpool</td> </tr> </table>			PI: F. Bridges-Cline	Guest Researcher	LDP NIMH				OTHERS: W.E. Wilson	Research Psychologist	DRG NIH	M. Radke-Yarrow	Chief	LDP NIMH	D. Hay	Research Psychologist	Univ. of London	T. Cox	Psychiatrist	Univ. of Liverpool
PI: F. Bridges-Cline	Guest Researcher	LDP NIMH																		
OTHERS: W.E. Wilson	Research Psychologist	DRG NIH																		
M. Radke-Yarrow	Chief	LDP NIMH																		
D. Hay	Research Psychologist	Univ. of London																		
T. Cox	Psychiatrist	Univ. of Liverpool																		
COOPERATING UNITS (if any) Division of Research Grants, NIH University of London University of Liverpool																				
LAB/BRANCH Laboratory of Developmental Psychology																				
SECTION																				
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892																				
TOTAL MAN-YEARS: 1.02	PROFESSIONAL: .50	OTHER: .52																		
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews																				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Experiences in the family are presumed to play a significant role in the socialization of the child, the development of his or her personality, and the emergence of any <u>pathological characteristics</u>. Of primary interest in this research is the functioning of different sorts of families with respect to a central dimension of family life, the provision of care to its members. An immediate objective of this study is the development of a <u>behavioral family interaction coding system</u> that focuses on the giving and receiving of care among family members. The goal is for the coding system to be at the same time sufficiently broad in the conceptualization of "caregiving" as to be applicable to family life in different cultures and family members of different ages, and sufficiently articulated as to be capable of differentiating families with and without varying kinds of internal and external stressors. An additional aim of the study is the examination of associations of <u>parental depression</u> and other <u>family stressors</u> with varying <u>patterns of family caregiving</u>. This aim derives from the view that family caregiving is a potential mediator of factors such as economic distress or parental pathology that place children at risk for later problems.</p>																				

Project Description

The effects of factors that place children at risk for later problems, such as a family's parental psychopathology or economic distress are likely to be mediated through changes in or dysfunctional patterns of, the provision of care to and by family members. An immediate objective of this study is the development of a family interaction coding system that focuses on the giving and receiving of care among family members. The system is to be sufficiently broad in the conceptualization of "caregiving" as to be applicable to family life in different cultures and family members of different ages, and sufficiently articulated as to be capable of differentiating families with and without varying kinds of internal and external stressors. The unit of analysis that has been selected for this coding system differs from that used in most other observational measures of family process. In contrast to a focus on the individual's action or trait, the emphasis is the events and states in family life to which members of the family may or may not respond, and the family members' perceptions of, and responsiveness to, each other's affective, behavioral, and cognitive states.

In addition, an aim is to examine and describe the varying patterns of caregiving that are associated with variations in family stressors and family background variables. Of primary interest is the factor of parental depression and the way in which this factor becomes manifest in the expression of and response to particular kinds of needs of family members. One question to be asked concerns the extent to which reversals of the usual caregiver role (e.g., an older sibling takes on the role of parent) occur in families with parental depression, and the extent to which it is harmful or beneficial for children to take on caregiving roles in their families.

Method

The major goal of the coding system is to describe how members of a family, parents and children alike, express their needs for care, react to each other's needs, and accept or rebuff whatever care is offered. The starting point for the description of a family's provision of care is the identification of needs that may be perceived or inferred by family members. The observation procedure requires four judgments: 1) determining whether a particular family member is in need of care at a particular time, and characterizing the ways in which the need manifests itself; 2) determining which, if any, of the other family members respond to that need, and characterizing the nature of their response; 3) characterizing the reaction of the person in need to the other family members' responses; and 4) recording instances in which one family member provides or ascertains the need for care for another in the absence of any visible need.

Two studies are involved: one is the NIMH Childrearing Study, and the other (to be referred to as the London Study) is conducted by Dr. Cox and his colleagues at the University of London. The two studies share some common research goals, as well as diverge in some aspects. Each examines families under stress, due to the affective illness of a parent (NIMH) and conditions associated with economic distress (London study). The issues addressed by each reflect concern about the extent to which very young children's experiences in the family

place them at risk or protect them from the likelihood of later disturbance.

Significance to Biomedical Research

Our goal in this study is to better understand the processes whereby affective illness in a parent may affect the child's experience and understanding of the giving and receiving of care in his relationships with others. Clearly, the primary social setting within which the child acquires knowledge and experience of caregiving is the family. The development of a robust observational measure of the family's pattern of caregiving will make available a widely applicable tool for researchers interested in studying significant dimensions of family environment that are associated with differential developmental outcomes.

Proposed Course

The coding system has been developed. Coding of the observational data from the families in the NIMH Childrearing Study has been completed. Analyses of these data in conjunction with other family level variables will be performed and a manuscript prepared.

Publications:

None



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02371-02 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Patterns of Alliance in Families With and Without Parental Depression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F. Bridges-Cline

Guest Researcher

LDP NIMH

OTHER: W.E. Wilson

Research Psychologist

DRG/NIH

COOPERATING UNITS (if any)

Division of Research Grants, NIH

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been incorporated into project Z01 MH 02370.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02372-02 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychiatric Status of Children of Depressed Parents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Radke Yarrow	Chief	LDP NIMH
OTHERS:	L. Cytryn	Medical Officer (Psych.)	LDP NIMH
	D. McKnew	Medical Officer (Psych.)	LDP NIMH
	L. Kuczynski	Associate Professor	Univ. Of Guelph
	T. Sherman	Research Psychologist	LDP NIMH
	E. Nottelmann	Statistician	LDP NIMH

COOPERATING UNITS (if any)

University of Guelph, Guelph, Ontario, Canada

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.05

PROFESSIONAL:

.30

OTHER:

1.75

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The offspring of unipolar depressed, bipolar depressed, and psychiatrically well parents were evaluated in terms of their relationships, their affective development and regulation, and their feelings and concepts about self. As part of a battery of measurements, psychiatric evaluations were made beginning at age 5, using a semi-standard interview (Child Assessment Schedule) plus mother's reports (Child Behavior Check List). The objective of this study is to investigate the status of the child as estimated by this data source. At this stage of analysis, each child was classified as manifesting one or more problems serious enough to be of clinical concern. In a cross-sectional analysis of the families seen thus far, the offspring of well, unipolar depressed, and bipolar depressed mothers were compared. Among the 5- to 6-year-olds, problems appeared in 8%, 39%, and 35% of the offspring in the well, unipolar, and bipolar groups, respectively. Among their siblings between 8 and 11 years of age, the comparable figures are 24%, 47%, and 36%. Among their older siblings in teens and young adulthood, the comparable figures are 25%, 65%, and 64%. Eight percent of the children of the well mothers had a problem (not necessarily the same problem) at both times; 20% of the children of the unipolar and of the bipolar mothers had a problem or problems of clinical concern at both periods. Based on the psychiatric interview, the groups were not clearly distinguished in content of problems.

Project Description:

The offspring of unipolar depressed, bipolar depressed, and psychiatrically well parents were evaluated in terms of their relationships, their affective development and regulation, and their feelings and concepts about self. As part of a battery of measurements, psychiatric evaluations were made beginning at age 5, using a semi-standard interview (Child Assessment Schedule) plus mother's reports (Child Behavior Check List). The objective of this study is to investigate the status of the child as estimated by this data source.

Methods and Major Findings:

Sibling pairs were seen in initial evaluations when the older child was between 5 and 8 years and the younger between 1 1/2 and 3 years. They were studied again three years later.

At this stage of analysis, each child was classified as manifesting one or more problems serious enough to be of clinical concern. In a cross-sectional analysis of the families seen thus far, the offspring of well, unipolar depressed, and bipolar depressed mothers were compared. Among the 5- to 6-year-olds, problems appeared in 8%, 39%, and 35% of the offspring in the well, unipolar and bipolar groups, respectively. Among their siblings between 8 and 11 years of age, the comparable figures are 24%, 47%, and 36%. Among their older siblings in teens and young adulthood, the comparable figures are 25%, 65%, and 64%.

To estimate continuity of problems, comparisons of problem status were made of those children who had been given the psychiatric interview between 5 and 8 years and again 3 years later. Eight percent of the children of the well mothers had a problem (not necessarily the same problem) at both times; 20% of the children of the unipolar and of the bipolar mothers had a problem or problems of clinical concern at both periods. The child problems revealed in the psychiatric interview include a range: problems of separation from mother, general anxiety, problems of mood regulation, oppositional behavior, attentional problems, physical disorders, and problems of aggression. The groups were not clearly distinguished in content of problems. This conclusion is likely to be altered, however, when the total battery of child assessments (behavioral observations, parents' and teachers' reports) are integrated with the child's interview data.

Proposed Course

A report of the findings on children's psychiatric status in relation to diagnostic status will be prepared. Additional analyses, not yet performed, will include data on the fathers' diagnostic status, and family history data.

Significance to Biomedical Research:

Research on the concordance of psychiatric problems of depressed parents and their offspring is mainly without information on course of development of offspring problems or on the conditions or processes underlying the development of offspring problems or well-being. The data from this study begin to provide such information.

Publications:

Radke-Yarrow M. Parental depression and parent-child interaction. In: Patterson GR, ed. Family social interaction: content and methodological issues in the study of aggression and depression. Hillsdale, NJ: Lawrence Erlbaum Associates, in press.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02380-02 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Stressful Life Events and Childhood Adjustment

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. Richters	Research Psychologist	LDP NIMH
OTHERS:	D. Pellegrini	Psychologist	Catholic University
	M. Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS (if any)

Catholic University

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.00

PROFESSIONAL:

.60

OTHER:

.40

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project explores the links between stressful life events affecting the family, and the social-emotional adjustment of the children. The analyses are based on data from families participating in the longitudinal study (Z01 MH 02144), which includes children of parents with and without a history of affective disorder. Stressful life events to which the children have been exposed are assessed through an interview with the mother.

The objective is to investigate the links between parental psychopathology, stressful life events, and the social-emotional adjustment of children. The analyses are based on data from families participating in the longitudinal study (Z01 MH 02144), which includes children of parents with and without a history of affective disorder (based on psychiatric SADS-L interviews). Data on stressful life events to which the children have been exposed are obtained in intensive, semi-structured interviews with mothers. Assessments of children's functioning are based on reports from parents and teachers, and observations of behavior with an unfamiliar peer. Families with a depressed parent (or parents) have significantly higher than average levels of marital distress, health problems, financial problems, housing problems, job difficulties, relationship problems and spouse maladjustment. Moreover, the depressed women evidenced significant levels of maladjustment and/or incompetence in coping with these problems. Differences between families with and without a depressed parent are sufficiently strong that knowledge of these aspects of family life allows us to correctly predict (via discriminant function analysis) the depressed or normal status of 84% of the families.

Project Description

The objective is to investigate the links between parental psychopathology, stressful life events, and the social-emotional adjustment of children.

Methods and Findings

The analyses are based on data from families participating in the longitudinal study (Z01 MH 02144), which includes children of parents with and without a history of affective disorder (based on psychiatric SADS-L interviews). Data on stressful life events to which the children have been exposed are obtained in intensive, semi-structured interviews with mothers. Assessments of children's functioning are based on reports from parents and teachers, and observations of behavior with an unfamiliar peer.

Stressful life events are classified by type; long- and short-term stress value; timing relative to the child's age; the roles played by each family member in causing, exacerbating or lessening the negative impact of each event. Coding of interviews was done by persons not familiar with the child's functioning.

Families with a depressed parent (or parents) have significantly higher than average levels of marital distress, health problems, financial problems, housing problems, job difficulties, relationship problems and spouse maladjustment. Moreover, the depressed women evidenced significant levels of maladjustment and/or incompetence in coping with these problems. Differences between families with and without a depressed parent are sufficiently strong that knowledge of these aspects of family life allows us to correctly predict (via discriminant function analysis) the depressed or normal status of 84% of the families.

A cumulative environmental risk index was created, ranging from 0 to 4: a psychiatrically ill mother, a psychiatrically ill father, a high level of stressful life events, and a high level of chronic family problems. All of the children who were rated by both teachers and mothers as having significant externalizing problems had 3 or 4 on the 4-point risk scale. When this analysis is extended to include all children for whom we have ratings from their mothers a similar pattern emerges. None of the children with a risk index of 0 was classified as having significant behavior problems; 11% of those with a risk index of 1, 31% of those with a risk index of 2, and 58% of those with a risk index of 3 or 4 were classified as having significant behavior problems relative to their peers.

Proposed Course

After the total sample has been interviewed and scored, analyses described above will be completed and manuscripts will be prepared for publication.

Significance to Biomedical Research

Although the link between stressful life events and psychopathology has been demonstrated over the years, the mechanisms through which they are related,

and their interrelated impact on children, are not well understood. This research will contribute to our understanding of these links.

Publications:

Richters J. Exposure to parent psychopathology and children's adjustment chronic versus episodic stress. In: Hahlweg K, Goldstein M, eds. Understanding major mental disorders: the contribution of family interaction research. New York: Plenum, 1987;74-90.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02381-02 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functioning of Depressed Mothers Within and Between Episodes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. Richters

Research Psychologist

LDP NIMH

OTHERS: M. Radke-Yarrow

Chief

LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.45

PROFESSIONAL:

.25

OTHER:

.20

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Are maternal child-related behavioral deficits characteristic of women with a history of depression, or are difficulties present only when mothers are experiencing an acute episode of depression? Are there certain patterns of aberrant child-related behaviors that wax and wane with depressive episodes, and others that reflect more or less enduring behavioral characteristics of depressed mothers? In this longitudinal study it is possible to compare maternal characteristics of two groups of mothers with a history of affective disorder: those who are in an episode and those who are between episodes of depression at the time of their participation in the study when they are observed with their children. Preliminary analyses indicate that depressed mothers who are currently in episode are not more likely than those who are not in episode to report behavior problems in their children. Both groups, however, report significantly more child behavior problems than mothers without a history of depression. Nor do depressed mothers who are in a self-reported depressed mood report higher levels of child behavior problems than depressed mothers who report non-depressed moods. These data argue against the likelihood that mothers' depressed moods or depressive states (episodes) lead to systematically distorted reports of problems in their children.

Depressed mothers experiencing an episode of depression, however, do report more distress in their marriages than depressed mothers who are not experiencing an episode of depression. Mothers' behaviors with and around their children while playing with a peer (an experimental laboratory situation) were compared. Depressed women in an episode of depression did not differ significantly from depressed women who were not in episode with respect to appropriateness of their interactions with their children.

Project Description:

Are maternal child-related behavioral deficits characteristic of women with a history of depression, or are difficulties present only when mothers are experiencing an acute episode of depression? Are there certain patterns of aberrant child-related behaviors that wax and wane with depressive episodes, and others that reflect more or less enduring behavioral characteristics of depressed mothers? In this longitudinal study it is possible to compare maternal characteristics of two groups of mothers with a history of affective disorder: those who are in an episode and those who are between episodes of depression at the time of their participation in the study when they are observed with their children.

Methods:

Analyses are based on data drawn from families participating in the longitudinal study (Z01 MH 02144) of child development and child rearing in families with and without a history of affective disorder. Assessments of parental psychopathology are based on psychiatric (SADS-L) interviews. To date we have studied 28 mothers with no history of affective disorder, 33 mothers with a history of affective disorder who were not experiencing a depressive episode when they were observed, and 24 mothers with a history of affective disorder who were experiencing a depressive episode at the time of assessment. Mothers and their children were observed in a variety of situations within a laboratory apartment designed to approximate natural rearing conditions. Mothers are being compared on symptom-related behaviors and on interactions with their children, including patterns of affect such as anger and affection (Z01 MH-02207), patterns of content of verbal interactions (Z01 MH-02220), and methods of regulating child behavior (Z01 MH-02169).

Findings:

Preliminary analyses indicate that depressed mothers who are currently in episode are not more likely than those who are not in episode to report behavior problems in their children. Both groups, however, report significantly more child behavior problems than do mothers without a history of depression. Nor do depressed mothers who are in a self-reported depressed mood report higher levels of child behavior problems than depressed mothers who report non-depressed moods. These data argue against the likelihood that mothers' depressed moods or depressive states (episodes) lead to systematically distorted reports of problems in their children.

Depressed mothers experiencing an episode of depression, however, do report more distress in their marriages than depressed mothers who are not experiencing an episode of depression. Mothers' behaviors with and around their children playing with a peer (an experimental laboratory situation) were compared. Depressed women in an episode of depression did not differ significantly from depressed women who were not in episode with respect to appropriateness of their interactions with their children.

On the basis of the data analyzed thus far, it appears that the important differences between depressed and non-depressed mothers reported in the longitudinal study (see other project reports) may be relatively stable and characteristic and not uniquely a function of depressed episodes, per se.

Proposed Course:

Coding and analyses for the maternal behaviors described above are in varying stages. Report preparation will follow.

Significance to Biomedical Research:

Questions concerning the state/trait dependence of maternal behaviors are of crucial importance to an understanding of the links between maternal depression and offspring adjustment.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02408-01 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Assessing Competencies of Children at Risk for Psychiatric Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: K. Free Research Psychologist LDP NIMH

OTHERS: E. Nottelmann Statistician LDP NIMH

M. Radke-Yarrow Chief LDP NIMH

W. Habelow Research Psychologist LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.40

PROFESSIONAL:

.20

OTHER:

.20

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There has been increasing attention focused on children who manage to cope competently despite expected vulnerability to psychological and psychiatric disturbance. The study of competencies in these children is viewed as important both to aid in the understanding of the developmental processes of children at risk as well as to formulate intervention strategies based on their strengths. The search for determinants and correlates of psychopathology and mental health has lead researchers to examine prospectively children who are at risk for developing emotional problems. Although children at risk have been found to develop some form of psychological disturbance more often than children who were not at risk, there are children deemed at risk who appear to develop normally. The present study focuses on these children.

Project Description:

There has been increasing attention focused on children who manage to cope competently despite expected vulnerability to psychological and psychiatric disturbance. The study of competencies in these children is viewed as important both to aid in the understanding of the developmental processes of children at risk as well as to formulate intervention strategies based on their strengths. The search in psychology and psychiatry for the determinants and correlates of psychopathology and mental health has lead many researchers to examine prospectively children who are at risk for developing emotional problems. Although children at risk have been found to develop some form of psychological disturbance more often than children who were not at risk, there are children deemed at risk who appear to develop normally. The present study focuses on these children.

The purpose of the present protocol is (1) to begin to operationalize the construct of competency; (2) to determine the competencies of a sample of children at risk for psychiatric disorder; (3) to examine the development of these competencies from a longitudinal perspective and to begin to predict future behavior from information about already acquired competencies.

Methods:

The subjects for this study are participants in the larger Childrearing Project (Z01 MH 02155); this includes mother, father, and two sibling children, each of whom has been evaluated for psychiatric disturbance.

The first stage of this project is to organize the indicators of child competencies that appear in the collected data in the longitudinal study. These are self-report data (from the Child Assessment Schedule [CAS]) and mother-report data (from the Achenbach Child Behavior Check List). The analysis will include those items which pertain to characteristic strengths and competencies in the child, i.e., social competence -- friendships, family relationships; physical competence -- hobbies, sports activities; cognitive competence -- school achievement. Competency on the part of the child will be investigated as it corresponds with depressive symptomatology, or other forms of psychopathology, in the parents.

Proposed Course:

In conjunction with this data analysis, there will be an effort to define and operationalize the concept of "competency" in childhood. This will involve a review of the assessment instruments which have been used in other studies. A coding scheme will be developed to be applied to the observational data in the longitudinal study. The behavioral indices will be compared with the report data and will be investigated across the three assessment periods in the longitudinal study.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02409-01 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Differential Development of Siblings in Shared and Nonshared Family Environments

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. Nottelmann	Statistician	LDP NIMH
OTHERS:	M. Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.40

PROFESSIONAL:

.10

OTHER:

.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Children in the same family often differ significantly in psychological characteristics and behavioral outcomes. They also show significant resemblances. Although these facts are well known, the processes by which family environments are linked to sibling differences and similarities are only partly understood. Such understanding is critical to developmental theory and to theories of gene-environment interactions. The purpose of this study is to investigate the shared and nonshared family experiences of siblings in relation to their similar and different social and emotional development. The research has three objectives. The first is descriptive -- what are the family experiences of siblings? The second concerns the links between siblings' shared and nonshared family experiences and the developmental course of each sibling. The third is to investigate child-specific attributes (temperament, gender, age, and others) that interact with environment and with environmental influences on sibling development. This study is at a beginning stage.

Project Description:

Children in the same family often differ significantly in psychological characteristics and behavioral outcomes. They also show significant resemblances. Although these facts are well known, the processes by which family environments are linked to sibling differences and similarities are only partly understood. Such understanding is critical to developmental theory and to theories of gene-environment interactions. The purpose of this study is to investigate the shared and nonshared family experiences of siblings in relation to their similar and different social and emotional development. The research has three objectives. The first is descriptive -- what are the family experiences of siblings? The second concerns the links between siblings' shared and nonshared family experiences and the developmental course of each sibling. The third is to investigate child-specific attributes (temperament, gender, age, and others) that interact with environment and with environmental influences on sibling development.

Methods and Findings:

Mothers and children participating in the longitudinal study (MH-02155) have been observed in a laboratory apartment when the two children were 2 to 3 years and 5 to 8 years of age, and again three years later. The scripted experiences for the 2- to 3-hour sessions were intended as an analogue of ordinary day-to-day family routines. Videotaped observations of mother-child, child-child, father-child, and total family interactions permit comparisons of the experiences and responses of the two children. Other data sources provide independent assessments of the children. Coding and analysis have just begun.

Proposed Course:

This study is at a beginning stage. The necessary development of coding and analysis schemes will be undertaken.

Significance to Biomedical Research:

In studies in behavior genetics, the shared and nonshared environments of twins and nontwin siblings have generally been assumed and rarely measured. Empirical data documenting the environments of each child should contribute to the understanding of similarities and differences in the outcomes of children from the same families and to theories of gene environment interaction.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02410-01 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Stability and Change in Mother-Child Relationships

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.D. Nottelmann

Statistician

LDP NIMH

OTHER: M. Radke Yarrow

Chief

LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.50

PROFESSIONAL:

.30

OTHER:

.20

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Stability and change in mother-child relationships are examined in early childhood across a three-year interval, in mother-child dyads with mothers with a diagnosis of bipolar depression and mothers with a diagnosis of unipolar depression, in comparison with normal control mother-child dyads. How mother and child relate to each other is observed in a variety of videotaped situations when the children were between 2 to 3-1/2 years old and, again, about three years later, when the children were about 5 to 6 1/2 years old.

Project Description

Stability and change in mother-child relationships are examined in early childhood in three samples of mother-child dyads: (a) dyads with mothers with a diagnosis of bipolar depression, (b) dyads with mothers with a diagnosis of unipolar depression, and (c) dyads with well mothers. The way mothers and children relate to each other is observed in a variety of situations videotaped when the children were between 2 to 3-1/2 years old and, again, about three years later, when the children were about 5 to 6 1/2 years old.

Measurement of an interpersonal relationship requires assessment of both participants. Rarely, however, in evaluating parent and child interaction, are general mutuality or "fit" of the interactions assessed. Thus, most analytic systems record mother's behavior (her commands, encouragements, affection, etc.) and the child's responses (compliance, increased motivation, affection, etc.). In the present system, we are viewing the continuous behavior of both mother and child in identical terms of attending to and receiving from each other. In this way, it may be possible to observe child influences, as well as mother influences, on the relationship. Of special interest, in these terms, is the relationship of affectively ill mothers and their young offspring, as that relationship develops over time.

Method

The sessions in which mother-child relationships are examined were scripted to include situations typically encountered on a day-to-day basis by mothers and their children. They include goal-structured, semi-structured, and unstructured time, social time, and time during which mothers are asked to make themselves unavailable to their children.

The mother-child relationship is examined separately from the perspective of mother and child. Each situation is broken into segments that define sequentially (with duration) how time is used by the mother and how it is used by the child; namely (a) mother (or child) focuses attention on the other; (b) mother (or child) is busy with task demands (or play); and (c) mother (or child) is, by choice, focusing on some other activity. Three modes of relating are examined: verbal, visual, and physical; the degree to which each mode of interaction is used in a segment is rated (none, low, moderate, high). Also recorded for each segment are level of positive and/or negative affect expressed by mother and child.

In addition to the segment-by-segment evaluations, overall ratings are made for each situation that focus on reciprocity in interactions for verbal initiations, instrumental help, emotional support, and information. Also rated are the degree to which mother and child monitor each other (vocally and visually), their energy level (vocal, physical), responses to the other's withdrawal, problems of mutuality (intrusive and/or aloof behaviors) in their relationship, and degree of overall dyadic harmony.

Significance to Biomedical Research

The relationship of mother and child in the formative years is believed to be important in shaping the child's social, emotional, and cognitive development. Impairments in this relationship are assumed to place the child at risk for the development of problems. The data from this study will aid in identifying significant indicators of problems in the depressed mother-child relationship as it develops progressively from 2 to 5 years.

Proposed Course

Coding and descriptive data analysis should be completed. Statistical analyses should be in progress within the year.

Publications

NONE

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02411-01 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sleep Disturbances in Young Children of Mothers with An Affective Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.D. Nottelmann

Statistician

LDP NIMH

OTHERS: S. Stoleru

Chef de Clinique-Asst. General Henrion
Bertier, France

M. Radke Yarrow

Chief

LDP NIMH

COOPERATING UNITS (if any)

General Henrion Bertier, Nevilly-Sur-Seine, France

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.40

PROFESSIONAL:

.20

OTHER:

.20

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The incidence and stability over time of sleep disturbance, based on mothers' reports, are examined in children whose mothers have a diagnosis of bipolar depression, children whose mothers have a diagnosis of unipolar depression, and well mothers. Mothers' reports were obtained twice on two of their children: (a) on the younger children when they were between 2 and 3 1/2 years old and again when they were between 5 and 6 1/2 years old; and (b) on the older children when they were between 5 and 8 years old and again when they were between 8 and 11 years old.

Project Description

The incidence and stability over time of sleep disturbance, based on mothers' reports, are examined in children whose mothers have a diagnosis of bipolar depression, children whose mothers have a diagnosis of unipolar depression, and well mothers. Mothers' reports were obtained twice on two of their children: (a) on the younger children when they were between 2 and 3 1/2 years old and again when they were between 5 and 6 1/2 years old; and (b) on the older children when they were between 5 and 8 years old and again when they were between 8 and 11 years old.

Method and Preliminary Findings

Information on children's sleep problems (i.e., "nightmares," "sleeps less than most children," and/or "trouble sleeping") was obtained from mothers' ratings of behavior problems on the Child Behavior Checklist. In order to determine if mothers who have an affective disorder generally have a tendency to over- or under-report behavior problems, report of sleep problems is being compared with of two other 3-item sets of problems: physical problems (e.g., "constipated, doesn't move bowels") and behavior problems (e.g., "lying or cheating").

One item considered for physical problems, "headaches," is being replaced, as it was reported primarily by affectively ill mothers. That is, of the 16 mothers who reported headaches for one or both of their children at the first and/or second assessment, 7 had a diagnosis of bipolar depression, 5 had a diagnosis of unipolar major depression, 2 had a diagnosis of unipolar minor depression, and 2 were well mothers.

Disturbance in sleep, but not in physical or behavior problems, was found significantly more often at Time 1 in the younger child (age range 2 to 3 1/2 years) and the older child (age range 5 to 8 years) of mothers with a diagnosis of unipolar depression than in children of well mothers. At Time 2 (younger child's age range 5 to 6 1/2 years; older child's age range 8 to 11 years), disturbance in sleep was found significantly more often only in the younger child of mothers with a diagnosis of unipolar depression than in children of well mothers. Again, at Time 2, nonsignificant differences in physical and behavior problems between children of affectively ill mothers and children of well mothers suggest that the sleep disturbance findings are not due to over- or under-reporting by affectively ill mothers.

Significance to Biomedical Research

Recent studies have shown that children of depressed parents are at risk for psychopathology. Sleep disturbance tends to be a manifestation of psychopathology in young children.

Proposed Course

Data analysis and manuscript preparation should be completed within the year.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02412-01 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Correspondence Between Mothers' Self-Reported and Observed Childrearing Practices

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Kochanska Research Psychologist LDP NIMH

OTHERS: L. Kuczynski Associate Professor Univ. of Guelph
M. Radke-Yarrow Chief LDP NIMH

COOPERATING UNITS (if any)

University of Guelph, Guelph, Ontario, Canada

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.50

PROFESSIONAL:

.30

OTHER:

.20

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Correspondence between mothers' reports, regarding their parenting attitudes, measured by the Block Childrearing Practices Report (Q-Sort) and their actual rearing practices was examined in 68 mothers of young children. The child-Block Q-Sort factors were selected and combined to represent authoritarian/restrictive and authoritative/democratic patterns of parenting attitudes in broader sense. Actual maternal childrearing practices were observed during 90 minutes of naturalistic interactions between the mothers and their children. Maternal verbal and physical control techniques and the children's responses to control (cooperation or resistance) were coded. The authoritarian pattern of attitudes was positively associated with the use of direct commands, physical enforcements, reprimands, and prohibitive interventions, and negatively associated with the use of indirect suggestions. The authoritative pattern was positively related to the use of indirect suggestions and positive incentives, and negatively related to the use of physical enforcements, prohibitive interventions and direct commands. The two affective attitudes, measured by the Block Q-Sort: enjoyment of parental role and negative affect towards child, seemed to be more a result of child's cooperation/resistance during the interaction than predictors of mothers' control strategies.

Project Description

Objectives

Despite numerous research efforts, the correspondence between verbally expressed parental attitudes and actual childrearing behaviors has been difficult to establish. The purpose of this study was to examine the relations between 68 mothers' reports and their actual control practices directed to their young children (16 to 44 months). It was expected that the correspondence between reports and behavior would be found when broadly conceptualized patterns of attitudes relating to discipline (authoritarian and authoritative), and maternal behaviors across variety of naturalistic contexts are compared. It was also expected that verbally expressed affective attitudes towards the child (enjoyment and negative affect) would be associated with the child's cooperation/resistance during the interactions.

Methods Employed

Maternal childrearing attitudes were assessed with the use of the Block Child-rearing Practices Report (Q-Sort). Their actual discipline strategies were examined during 90 minutes of naturalistic interactions. Every maternal control intervention and child response in a variety of situations encompassing typical family routines and contexts was coded with the use of behavioral coding system (Z01 MH 02152). Maternal discipline categories included: direct commands, indirect suggestions, reprimands, positive incentives, explanations, bargains, alternatives, physical enforcements, and prohibitive interventions. Child response categories included compliance and resistance (a composite measure of several forms of noncompliance).

Findings

There was a strong correspondence between broadly defined patterns of child-rearing attitudes and maternal observed behaviors. The endorsement of the authoritarian pattern was positively associated with the frequent use of direct commands, physical enforcements and prohibitions, and negatively associated with the use of polite suggestions. The authoritative pattern, expressed in the verbal report, was positively associated with the use of polite suggestions and positive incentives, and negatively related to the use of direct commands, enforcements, and prohibitions. As predicted, maternal affective attitudes regarding the child were associated with child's compliance during interaction.

Significance for Biomedical Research

Verbal reports regarding mothers' childrearing attitudes and their feelings about the children are very commonly used in developmental and clinical research on socialization, both in normal, and in high-risk populations. Understanding the complexity of the relations between maternal attitudes regarding discipline, feelings about the child, maternal control behaviors, and child behavior during interactions is necessary for better description of childrearing environment.

Proposed course

The manuscript has been submitted to a journal and its revised version is currently under review. Project is closed.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02413-01 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Emotion Language in Young Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. Zahn-Waxler	Chief, Section on Child Behavior Disorders	LDP/NIMH
Others:	P. Cole S. Denham	Senior Staff Fellow Associate Professor	LDP/NIMH George Mason Univ.

COOPERATING UNITS (if any)

George Mason University, Fairfax, Virginia

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.33

PROFESSIONAL:

.30

OTHER:

.03

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this research is to identify conditions in early development that influence children's ability to communicate about emotions. Mothers' specific communication patterns, personality characteristics and affective state (depressed vs. well) were examined. One hundred fifty-five mothers and their 2- to 4-year-olds were studied. Emotion language was based on a standard sample of communication generated by exposure to a book of photographs of infants' displaying expressions of various positive and negative emotions. Strong patterns of correspondence between mothers' and children's communications about emotions were identified. The more often mothers discussed positive and negative emotions, used evaluative language, described causes and reasons, and provided explanations for feelings -- the more their children did so. The ways mothers talked about emotions with their children were embedded in broader personality patterns, e.g., open and perceptive mothers talked more frequently and freely about emotions. Depressed mothers were less able than well mothers to adapt their communications to the needs and capacities of the child. Moreover, depressed mothers tended to avoid providing explanations for emotions.

Project Description

The ability to talk about emotions and feelings begins shortly after children first acquire language. As early as the second and third year of life, children begin (a) to discuss past and future emotions and (b) to talk appropriately about causes and consequences of emotional states. These communications serve adaptive purposes of informing others about children's inner lives and expressing awareness of others' internal states. The purpose of this research is to identify early conditions that facilitate or impede children's capacities for talking about emotions. The conditions studied are mothers' specific communications with their children about emotions, as well as variations in mothers' affective state and personality.

Methods Employed and Major Findings

A standard procedure was developed to elicit discussions about emotions in mother-child interactions. Two samples were studied: (1) 51 mothers with two- to three-year-old children and (2) 104 mothers with two- to four-year-old children. Mothers in sample 1 were from a normal volunteer sample and were given the MMPI to assess personality characteristics. Mothers in sample 2 were screened and were diagnosed as having unipolar depression, bipolar depression or no psychopathology. Each mother-child dyad was given a book to view and discuss that contained eight photographs of infants' displaying expressions of various positive and negative emotions. The session was videotaped and language patterns of mother and child were transcribed later from the videotapes.

In both studies there was evidence of considerable variability in the frequency and styles of emotion language. Strong patterns of correspondence between mothers and children's communications about emotions were identified. The more often mothers discussed positive and negative emotions, used evaluative language, described causes of emotions, and provided explanations for feelings -- the more their young children did so. Mothers who were highly accurate in their interpretations of others' emotions had children who were particularly accurate. Mothers who could be characterized as open and perceptive were especially likely to talk with their children about emotions, while mothers who were more defensive spoke less to their children about feelings. Mothers spoke more to older children than younger children about emotions and the possible causes of emotion, but this developmental pattern characterized only the well mothers. Depressed mothers, in contrast, did not adapt their communications to fit the increased communicative capacities and cognitive comprehension of their older children. Rather, they were twice as likely as well mothers simply to label (but not explain) negative emotions with both their younger and older children. In separate assessments, depressed mothers were more likely to see negative emotions (fear, anger and sometimes sadness) and less likely to see positive emotions (joy) in facial expressions of infants in situations of ambiguity.

Proposed Course

A report has been prepared for publication and another is being written. The work is being presented at the International Conference on Infant Studies, April '88. This is a final report.

Significance to Biomedical Research

This research identifies some factors that influence the child's awareness of and communication about emotions in self and others. Deficiencies in this area pose problems for social-emotional development. Basic research on processes associated with early affective communication patterns contribute to understanding of one aspect of affective development.

Publications

Zahn-Waxler C, Ridgeway D, Denham S, Usher B. Research strategies for assessing mothers' interpretations of infants' emotions. In: Emde R, Osofsky J eds. Parental Perceptions of Infant Emotions. Clinical Infant Report Series, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02415-01 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Longitudinal Psychiatric Status: Parent, Offspring

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Brown Medical Officer LDP/NIMH

Others: K. Free Staff Fellow LDP/NIMH
M. Radke-Yarrow Chief LDP/NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Developmental Psychopathology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.60

PROFESSIONAL:

.50

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aggregation of problems in adult offspring of depressed parents has been widely documented. In this project, the focus is on the early manifestations of problems and the developmental course of problems in the offspring of depressed parents. As part of a longitudinal study, this analysis follows investigation of still earlier childhood manifestations of behavioral disorders (MH-02372).

The families who have been studied in the two earlier assessment periods are the participants. They were seen initially when two siblings in each family were 2 years and 5 to 8 years of age; they were followed 3 years later, and they are now returning 3 years later for a third time. Psychiatric status of mother and father is reevaluated based on standard psychiatric interviews, the Schedule for Affective Disorders and Schizophrenia Interview (SADS-I), the Structured Clinical Interview for DSM-III-R (SCID) and the Personality Disorder Examination (PDE). Psychiatric status of each child is also reevaluated based on a standard psychiatric interview, the Diagnostic Clinical Interview for Children and Adolescents (DICA-R). Further to assess the child, mother and father are interviewed using the DICA-P(R). Medical and biological assessments of the children are anticipated.

Project Description:

The aggregation of problems in adult offspring of depressed parents has been widely documented. In this project, the focus is on the early manifestations of problems and the developmental course of problems in the offspring of depressed parents, contrasted to those seen in the offspring of parents without an Axis I psychiatric diagnosis (DSM-III-R). Personality Disorder assessments are being added to parental assessment in the upcoming reassessment (time 3).

Methods:

The families who have been studied in the two earlier assessment periods are the participants. They were seen initially when two siblings in each family were 2 years and 5 to 8 years of age; they were followed 3 years later, and they are now returning 3 years later for a third time. Psychiatric status of mother and father is reevaluated based on standard psychiatric interviews, the Schedule for Affective Disorders and Schizophrenia - Interval (SADS-I), the Structured Clinical Interview for DSM-III-R (SCID), and the Personality Disorder Examination (PDE). Psychiatric status of each child is also reevaluated, based on a standard psychiatric interview, the Diagnostic Clinical Interview for Children and Adolescents (DICA-R). Further to assess the child, mother and father are interviewed using on the DICA-P(R). Medical and biological assessments of the children are anticipated.

Each interviewer of parents and children is blind to all other information about the family. These data, in addition to providing one major assessment of children's outcomes in middle and later childhood, will be part of an integrative analysis involving concurrent projects by other investigators in which (a) environmental variables in home and school are measured, (b) other behavioral and interview evaluations of the children are made, and (c) standard medical and clinical laboratory assessments are made. Measures of peptides associated with growth and serotonergic measures that may be associated with aggressive and depressive behaviors, sleep disturbance, suicidal ideation, and headaches in children will also be assessed, in keeping with some of the research interests described in Z01 MH 00183-03 LDP (Dr. Brown).

Significance to Biomedical Research:

This longitudinal study provides prospective data on the development of offspring of depressed parents in later childhood. The offspring research has generally not had either the data on these periods of development or data on the developmental course of children of depressed parents. Inclusion of personality assessments of parents is timely in that such assessments are becoming widely recognized as important trait variables, not only in clinical psychiatry but also with regard to biological variables. The effect of parental traits on offspring may be no less significant than that attributed to clinical states in parents. The relation between such adult personality variables and offspring characteristics has been a minimally researched area at this time.

Proposed Course:

Psychiatric assessments will begin as soon as piloting and training have been completed.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02416-01 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology and Behavior of Aggression and Suicide

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Brown

Medical Officer

LDP/NIMH

Others: M. Linnoila

Chief

LCS/NIAA

J. Kleinman

Deputy Chief, Clinical

St. Elizabeth's

Brain Disorders Branch

Hospital/NIMH

D. Murphy

Chief

LCS/NIMH

J. Rapoport

Chief

CHP/NIMH

F. Goodwin

Director

DIRP/NIMH

COOPERATING UNITS (if any)

LCS/NIAA

St. Elizabeth's Hospital/NIMH

LCS/NIMH; CHP/NIMH; DIRP/NIMH

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Developmental Psychopathology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

44

PROFESSIONAL:

34

OTHER:

10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies that relate human aggression (including hyperactivity and conduct disorder in children) and suicide to various behavioral and biological factors have been ongoing. Some of the most significant findings have included pharmacokinetic and metabolic studies of amphetamine administered to hyperactive and conduct disordered children, and a trivariate relation among a history of aggressive behavior, a history of suicidal behavior, and lower cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5HIAA). Data indicate that certain aggressive, impulsive, and depressive characteristics in childhood are inversely related to CSF 5HIAA measured during late adolescence. Family instability (particularly, alcoholism in a parent) during childhood is also associated with an increased likelihood of suicidal behavior in adolescence. These data, along with the work of other investigators studying aggressive and depressive behavior in childhood, indicate the possibility of traits associated with disordered serotonin metabolism; further, the less consistent relation between lower CSF 5HIAA and suicidal behaviors vs. aggressive behaviors, may indicate that some suicidal behaviors are a self-destructive manifestation of a more basic destructive (aggressive/impulsive) trait.

This is a continuation of project Z01 MH 00183.

Project Description:

Objectives: This report summarizes a group of interrelated collaborative studies. An objective is the investigation of the central nervous system (CNS) of children, adolescents, and adults with special reference to maturational changes and neuropsychiatric disorders as they relate to aggression, suicide, obsessive-compulsive disorder, and depression. Compared to research knowledge of biochemical-behavioral interactions in adult neuropsychiatry, less is known regarding neuropsychiatric disorders of children. In hyperactivity in children, an overly active catecholaminergic system was first advanced. Later, a functional deficiency in catecholamines was proposed, with the greater focus on a functional dopamine (DA) rather than norepinephrine (NE) deficiency. Alterations involving serotonin (5HT), acetylcholine (ACH) and phenylethylamine (PEA) have also been proposed. No single neurotransmitter system has been shown to have an etiological role. Indirect pharmacologic evidence has linked amine systems with adult psychiatric illness (particularly affective illness and schizophrenia). A purpose of this project is to extend the studies of CNS amines into larger and more diverse populations of psychiatric patients, and to assess biochemical-behavioral relations and whether such findings are diagnostically specific. Searching for interrelations between central biochemical functioning and repeated behavioral patterns may be as important as searching for traditional diagnostic specificity of biochemical findings. Confirmation of relations between CNS biochemistry and behavior could lead to more specific pharmacological treatments. Data have begun to accumulate on CNS biochemical function in the various personality disorders; i.e., the "aggressive-impulsive" personalities and OCD, criminality, aggressive/impulsive characteristics have been linked to a genetic determinant. Other patterns of behavior often seen within personality disorders -- depression, alcoholism, suicide, and obsessiveness -- also appear to have genetic components. Data from animals strongly suggest a relation between aggressive behavior and neurotransmitters, particularly 5HT. Drs. Frederick Goodwin and Marian Yarrow provide overall scientific supervision of this multi-faceted research effort.

Methods Employed:

A. Children and adolescents in inpatient and day-patient programs are assessed to define samples of ADD/CD, OCD, and OD. Children are evaluated by medical, psychiatric, psychometric examinations, and clinical laboratory studies. Medically indicated procedures are provided directly or by referral. Clinical, observational and behavioral rating instruments are utilized. Further details of this program can be found in Protocol #85-M-115 of Dr. Judith Rapoport. Pharmacological study results implemented in this program are briefly summarized below.

B. NIMH-Navy lumbar puncture (LP) studies have been described in detail in a previous annual report (Z01 MH 00092-11 BP). Family studies are under way in these subjects as well (Dr. Linnoila). LP's are being performed in children and adolescents who are aggressive/impulsive or compulsive (Dr. Kreusi). Blind clinical evaluations are being performed by Dr. Brown.

Among those individuals incarcerated for murder, responses to glucose tolerance test (GTT) and similar artificial sweetening will be assessed by the Thematic Apperception Test (TAT) along with baseline LP's in collaboration with Dr. Linnoila and colleagues. Measures of Y-chromosomal material may also be introduced. A further study involves an assessment of 5HT and its metabolites from autopsy material and LP's in conjunction with their clinical inpatient records in those individuals with a history of suicide and/or violence (in collaboration with Dr. Kleinman). 5HT variables in blood in aggressive/impulsive behavior is also being assessed (Dr. Murphy).

Major Findings:

Previous findings have been summarized in Z01 MH 00183-02 BP. New findings are summarized below.

1. As part of Dr. Brown's collaborative role in making clinical assessments of aggressivity-impulsivity in children and adolescents, in collaboration with Drs. Kreusi and Rapoport, he remains blind to CSF 5HIAA data that have now been collected on approximately 30 subjects. Further refinements in both content and reliability of inter-rater assessments are ongoing. In the NIMH-Navy research a negative correlation was found between CSF 5HIAA (in the Navy men) and a childhood history of ADD/CD, particularly in items related to aggressive and depressive affect. Medical history includes headaches in childhood. These findings have been further pursued in terms of suicidal behavior and family history of various kinds of stresses and instability. Data have now been analyzed that show that those individuals with a higher score for aggressive/impulsive behaviors had no greater mean scores on items related to family instability, stress, and loss than those with lower scores for aggressive/impulsive behavior, whereas higher family instability (particularly alcoholism in a parent) scores were associated with a history of suicide attempt as a late adolescent. Disturbed family history per se was not related to CSF 5HIAA, possibly indicating that various kinds of disturbed personality and behavior (present in all subjects studied) not of an aggressive and/or suicidal character, may also not be associated with 5HT metabolism, or at least not in the same way as that seen for aggressive/impulsive/suicidal individuals. These findings are consistent with other data in the literature that would support an inverse relation between aggressive/impulsive behavior or behavior thought to indicate dyscontrol and disinhibition as trait characteristics and CSF 5HIAA or other measures related to 5HT metabolism. Further, evidence seems to support aggressive/impulsive behavior being more clearly related to 5HT metabolism than a history of suicide attempts, and that suicidal behavior may be a form of aggressive behavior. The review of histories of schizophrenics for aggressive/suicidal behavior with LP and autopsy material is ongoing.

Significance to Mental Health Research:

Though childhood neuropsychiatric disorders have been considerably studied in the last few years, many diagnostic, psychopharmacological, and psychobiological questions are yet to be addressed. One avenue to ascertaining possible neuropathology is to understand more clearly the mechanisms of action of pharmacological compounds which effectively alter the

clinical conditions under study. The relation between such basic pharmacological knowledge and clinical effects has been under-studied in children in general. More importantly, for the future, basic biological factors in childhood neuropsychiatry which might elucidate the psychopharmacological responses are still only hypotheses.

Human suicidal behavior has enormous public health and social significance. Previously it has largely been studied from psychological and sociological points of view. Studies of animal models, as well as Gilles de la TOURETTE'S SYNDROME, ADD/CD in children, and prisoners suggest a relation between CNS neurotransmitter systems and aggressive behavior. The study of suicidal behavior in children is important directly for those affected children and as a predictor of later suicidal attempts. Both aggressive and suicidal problems are increasing. These studies lead to the possibility of identifying those at risk for anti-social and suicidal behaviors and possibly altering those behaviors through both neuropharmacological interventions as well as psychosocial interventions. The neurobiological aspects of alcoholism, either predisposing, concomitant, or resultant, are also of timely significance.

Proposed Course of Project:

The active data collection has been completed in the Navy collaborative project. Some neurochemical, behavioral, and psychological data are yet to be analyzed and reported. A follow-up may be undertaken. Additionally, collaboration continues on LP and autopsy studies of schizophrenics within the IRP at St. Elizabeth's Hospital. Collaboration continues also with the Child Psychiatry Branch (CPB) IRP.

Publications:

Brown GL, Goodwin FK. Overview of biological factors in suicide. In: The Task Force on Youth Suicide. Washington, D.C.: Department of Health and Human Services, in press.

Brown GL, Goodwin FK. Risk factors in suicidal behavior, Psychiatr Lett, in press.

Brown GL, Goodwin FK. Measurement of human aggression/impulsivity. In: Linnoila M, van Braag H, eds. Biological factors in human aggression. ACNP, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02417-01 LDP

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Longitudinal Study of Aggression and Social Competence in Young Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Zahn-Waxler

Chief, Section on

LDP/NIMH

Child Behavior Disorders

Others: E. Cummings

Associate Professor

Univ. of West

Virginia

K. Rubin

Professor

Univ. of Waterloo

S. Denham

Associate Professor

George Mason Univ.

P. Cole

Senior Staff Fellow

LDP/NIMH

COOPERATING UNITS (if any)

University of West Virginia, Morgantown, West Virginia

University of Waterloo, Waterloo, Canada

George Mason University, Fairfax, Virginia

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.53

PROFESSIONAL:

1.50

OTHER:

.03

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The most common causes of referral to child mental health services are childhood behavior problems involving misconduct, aggression and non-compliance. Children with early problems in these areas are at risk for later social-emotional problems and psychiatric disorders. The purpose of this research is to identify in the first years of life, characteristics of children that predict problem behaviors at school age, especially problems with aggression. Forty-eight two-year-old children of depressed and well mothers were observed and assessed on multiple occasions, using naturalistic and experimental procedures. They were seen again at ages five and six. Preliminary analyses indicated strong patterns of continuity over time in problems with aggression, particularly in children with depressed mothers. Children who were out-of-control and intensely aggressive toward playmates at age two were seen as aggressive with playmates and described by their parents as antisocial at ages five and six. Observed social competence at age five could be predicted from earlier skills in modulating emotions in social relationships (e.g., using positive emotions as a means to initiate and maintain interactions).

Project Description

The most common causes of referral to child mental health services are childhood behavior problems involving misconduct, aggression and non-compliance. The majority of cases seen in treatment are diagnosed as conduct and oppositional disorders. Children who display early problems in these areas are at risk for psychiatric disorders and for life-long problems in their social relationships. One purpose of this research is to identify, in the first years of life, characteristics of children that predict problem behaviors by school age, especially problems with aggression. A second goal is to examine how these externalizing problems (aggression, conduct problems) relate to internalizing problems (sadness, anxiety, social withdrawal). Patterns of continuity and discontinuity over time in problem behaviors are examined separately in children from high and low risk environments. Risk is defined in terms of maternal depression, since this factor has been linked both with externalizing and internalizing problems in children.

Methods Employed and Major Findings

Forty-eight two-year-old children were seen in three 1 1/2 hour laboratory sessions spaced two weeks apart. Children of both normal and depressed mothers (SADS-L) were studied. Each child was exposed to a range of challenging conditions in order to evaluate social and emotional interchanges primarily in interactions with a familiar playmate, but also with mother and with an adult stranger. Assessments were made of the child's ability to sustain social play, compete adaptively for resources, negotiate problems, cooperate, cope with frustration without resorting to intense aggression, empathize, and solve hypothetical social problems. Maternal characteristics evaluated were sensitivity, supportive presence and quality of assistance, and techniques used to encourage cooperation and sustained social and task-oriented involvement with others. Children's social skills in peer interactions were assessed again at age five. Self-report data on childrearing practices and the marital relationship were also obtained. At age six the Childhood Assessment Schedule, a psychiatric interview, was used to obtain psychiatric evaluations of the children. The mothers completed the Achenbach Child Behavior Check List.

There are strong patterns of continuity over time in problems with aggression, particularly in children with depressed mothers. Children who at age six were perceived by their parents as antisocial and showing conduct problems had, as two-year-olds, appeared to be emotionally out-of-control and frequently engaged in intense aggression with their playmates. Analyses of observed aggression toward peers also indicate significant continuities between ages two and five, confirming mothers' reports. That is, children who were frequently aggressive toward peers at age two, particularly children who initiated many fights, were also likely to be aggressive at age five. Strategies endorsed by five-year-olds for resolving conflict differed for boys and girls of well and depressed mothers. Children of depressed mothers, especially girls, were likely to generate strategies that reflected submissiveness or appeasement (e.g., giving up a desired object). Boys of

depressed mothers generated many aggressive strategies as well. Characteristics of children that may protect them from later emotional problems are being examined, namely the role of affect in promoting and maintaining interaction (e.g., using positive emotions as a means to initiate and maintain interactions) and to refrain from inappropriate use of negative emotions such as anger and sadness.

Proposed Course

All data have been coded and data analyses continue. Several manuscripts are in preparation.

In a new protocol on aggression, anger regulation and social competence, the continuing goal is to identify early precursors of later aggression. This expands on the earlier work to include, in addition to the previously identified behavioral and psychological characteristics, biological characteristics (constitutional factors) of children and personality and behavioral characteristics of their parents.

Significance to Biomedical Research and the Program of the Institute

An aim of prevention research is to identify early in development those child and family factors that contribute to later childhood disturbances. Antisocial behavior in children has tended to come to the attention of professionals when children reach school age, but may have much earlier origins. If early identification of behavior problems can be made, more effective intervention procedures could be planned.

Also, tracing the developmental trajectories of young children who manifest behaviors phenotypically similar to adult behavioral disorders will help in understanding the interaction of constitutional and environmental variables in behavioral disorders.

Publications

Kruesi M, Rapoport J, Cummings E, Berg C, Flament M, Radke-Yarrow M, Zahn-Waxler C. Sugar and aspartame: A challenge study with preschool children, *Am J Psychiatry*, 1987;144(11):1487-90.



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